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(71) Applicant *(for all designated States except US)*: NOVARTIS AG [CH/CH]; Schwarzwaldallee 215, CH–4058 Basel (CH).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: RYALS, John, Andrew [US/US]; 303 Brittany Place, Cary, NC 27511 (US). LAWTON, Kay, Ann [US/US]; 8016 Crooked Chute Court, Raleigh, NC 27612 (US). UKNES, Scott, Joseph [US/US]; 1003 Pinedale Drive, Apex, NC 27502 (US). STEINER, Henry–York [US/US]; 1800–103 Avantridge Road, Raleigh, NC 27606 (US). HUNT, Michelle, Denise [US/US]; 361 Vickers Road, Chapel Hills, NC 27514 (US). FRIEDRICH, Leslie, Bethards [US/US]; 4208 Wyckford Place, Apex, NC 27502 (US).

(74) Agent: ROTH, Bernhard, M.; Novartis AG, Patent– und Markenabteilung, Lichtstrasse 35, CH–4002 Basel (CH).

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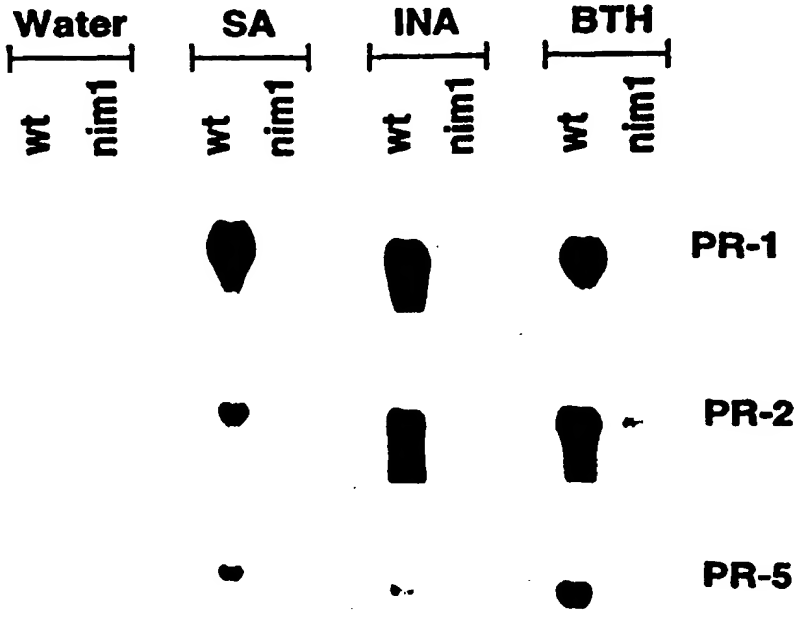
(54) Title: METHODS OF USING THE *NIM1* GENE TO CONFER DISEASE RESISTANCE IN PLANTS

(57) Abstract

The invention concerns the location and characterization of a gene (designated *NIM1*) that is a key component of the SAR pathway and that in connection with chemical and biological inducers enables induction of SAR gene expression and broad spectrum disease resistance in plants. The *NIM1* gene product is a structural homologue of the mammalian signal transduction factor IκB subclass α. The present invention exploits this discovery to provide altered forms of *NIM1* that act as dominant–negative regulators of the systemic acquired resistance (SAR) signal transduction pathway. These altered forms of *NIM1* confer the opposite phenotype as the *nim1* mutant in plants transformed with the altered forms of *NIM1*, i.e. the transgenic plants exhibit constitutive SAR gene expression and a constitutive immunity (CIM) phenotype. The invention further concerns transformation vectors and processes for overexpressing the *NIM1* gene in plants.



The transgenic plants thus created have broad spectrum disease resistance. The present invention further concerns DNA molecules encoding altered forms of the *NIM1* gene, expression vectors containing such DNA molecules, and plants and plant cells transformed therewith. The invention further concerns transformation vectors and processes for overexpressing the *NIM1* gene in plants. Disclosed are vectors and processes for producing overexpression of the *NIM1* gene in plants. The invention also concerns methods of activating SAR in plants and conferring to plants a CIM phenotype and broad spectrum disease resistance by transforming the plants with DNA molecules encoding altered forms of the *NIM1* gene product.

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METHODS OF USING THE *NIM1* GENE TO CONFER DISEASE RESISTANCE IN PLANTS

The present invention generally relates to broad-spectrum disease resistance in plants, including the phenomenon of systemic acquired resistance (SAR). More particularly, the present invention relates to the recombinant expression of wild-type and altered forms of the *NIM1* gene, which is involved in the signal transduction cascade leading to SAR to create transgenic plants having broad-spectrum disease resistance. The present invention relates further to high-level expression of the cloned *NIM1* gene in transgenic plants that have broad-spectrum disease resistance.

Plants are constantly challenged by a wide variety of pathogenic organisms including viruses, bacteria, fungi, and nematodes. Crop plants are particularly vulnerable because they are usually grown as genetically-uniform monocultures; when disease strikes, losses can be severe. However, most plants have their own innate mechanisms of defense against pathogenic organisms. Natural variation for resistance to plant pathogens has been identified by plant breeders and pathologists and bred into many crop plants. These natural disease resistance genes often provide high levels of resistance to or immunity against pathogens.

Systemic acquired resistance (SAR) is one component of the complex system plants use to defend themselves from pathogens (Hunt and Ryals, *Crit. Rev. in Plant Sci.* 15, 583-606 (1996), incorporated by reference herein in its entirety; Ryals et al., *Plant Cell* 8, 1809-1819 (1996), incorporated by reference herein in its entirety. *See also,* U.S. Patent No. 5,614,395, incorporated by reference herein in its entirety). SAR is a particularly important aspect of plant-pathogen responses because it is a pathogen-inducible, systemic resistance against a broad spectrum of infectious agents, including viruses, bacteria, and fungi. When the SAR signal transduction pathway is blocked, plants become more susceptible to pathogens that normally cause disease, and they also become susceptible to some infectious agents that would not normally cause disease (Gaffney et al., *Science* 261, 754-756 (1993), incorporated by reference herein in its entirety; Delaney et al., *Science* 266, 1247-1250 (1994), incorporated by reference herein in its entirety; Delaney et al., *Proc. Natl. Acad. Sci. USA* 92, 6602-6606 (1995), incorporated by reference herein in its entirety; Delaney, *Plant Phys.* 113, 5-12 (1997), incorporated by reference herein in its entirety; Bi et al., *Plant J.* 8, 235-245 (1995), incorporated by reference herein in its entirety; Mauch-Mani and Slusarenko, *Plant Cell* 8, 203-212 (1996), incorporated by reference herein in its

entirety). These observations indicate that the SAR signal transduction pathway is critical for maintaining plant health.

Conceptually, the SAR response can be divided into two phases. In the initiation phase, a pathogen infection is recognized, and a signal is released that travels through the phloem to distant tissues. This systemic signal is perceived by target cells, which react by expression of both SAR genes and disease resistance. The maintenance phase of SAR refers to the period of time, from weeks up to the entire life of the plant, during which the plant is in a quasi steady state, and disease resistance is maintained (Ryals et al., 1996).

Salicylic acid (SA) accumulation appears to be required for SAR signal transduction. Plants that cannot accumulate SA due to treatment with specific inhibitors, epigenetic repression of phenylalanine ammonia-lyase, or transgenic expression of salicylate hydroxylase, which specifically degrades SA, also cannot induce either SAR gene expression or disease resistance (Gaffney et al., 1993; Delaney et al., 1994; Mauch-Mani and Slusarenko 1996; Maher et al., *Proc. Natl. Acad. Sci. USA* 91, 7802-7806 (1994), incorporated by reference herein in its entirety; Pallas et al., *Plant J.* 10, 281-293 (1996), incorporated by reference herein). Although it has been suggested that SA might serve as the systemic signal, this is currently controversial and, to date, all that is known for certain is that if SA cannot accumulate, then SAR signal transduction is blocked (Pallas et al., 1996; Shulaev et al., 1995*Plant Cell* 7, 1691-1701 (1995), incorporated by reference herein in its entirety; Vernooij et al., *Plant Cell* 6, 959-965 (1994), incorporated by reference herein in its entirety).

Recently, Arabidopsis has emerged as a model system to study SAR (Uknes et al., *Plant Cell* 4, 645-656 (1992), incorporated by reference herein in its entirety; Uknes et al., *Mol. Plant-Microbe Interact.* 6, 692-698 (1993), incorporated by reference herein in its entirety; Cameron et al., *Plant J.* 5, 715-725 (1994), incorporated by reference herein in its entirety; Mauch-Mani and Slusarenko, *Mol. Plant-Microbe Interact.* 7, 378-383 (1994), incorporated by reference herein in its entirety; Dempsey and Klessig, *Bulletin de L'Institut Pasteur* 93, 167-186 (1995), incorporated by reference herein in its entirety). It has been demonstrated that SAR can be activated in Arabidopsis by both pathogens and chemicals, such as SA, 2,6-dichloroisonicotinic acid (INA) and benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) (Uknes et al., 1992; Vernooij et al., *Mol. Plant-Microbe Interact.* 8, 228-234 (1995), incorporated by reference herein in its entirety; Lawton et al., *Plant J.* 10, 71-82 (1996), incorporated by reference herein in its entirety). Following treatment with either INA or BTH or pathogen infection, at least three pathogenesis-related (PR) protein genes, namely, *PR-1, PR-2,* and *PR-5* are coordinately induced concomitant with the onset

of resistance (Uknes et al., 1992, 1993). In tobacco, the best characterized species, treatment with a pathogen or an immunization compound induces the expression of at least nine sets of genes (Ward et al., *Plant Cell* 3, 1085-1094 (1991), incorporated by reference herein in its entirety). Transgenic disease-resistant plants have been created by transforming plants with various SAR genes (U.S. Patent No. 5,614,395).

A number of Arabidopsis mutants have been isolated that have modified SAR signal transduction (Delaney, 1997). The first of these mutants are the so-called *lsd* (lesions simulating disease) mutants and *acd2* (accelerated cell death) (Dietrich et al., *Cell* 77, 551-563 (1994), incorporated by reference herein in its entirety; Greenberg et al., *Cell* 77, 551-563 (1994), incorporated by reference herein in its entirety). These mutants all have some degree of spontaneous necrotic lesion formation on their leaves, elevated levels of SA, mRNA accumulation for the SAR genes, and significantly enhanced disease resistance. At least seven different *lsd* mutants have been isolated and characterized (Dietrich et al., 1994; Weymann et al., *Plant Cell* 7, 2013-2022 (1995), incorporated by reference herein in its entirety). Another interesting class of mutants are *cim* (constitutive immunity) mutants (Lawton et al., 1993 "The molecular biology of systemic aquired resistance" in *Mechanisms of Defence Responses in Plants*, B. Fritig and M. Legrand, eds (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 422-432 (1993), incorporated by reference herein in its entirety). *See also,* International PCT Application WO 94/16077, both of which are incorporated by reference entirety herein in their entireties. Like *lsd* mutants and *acd2*, *cim* mutants have elevated SA and SAR gene expression and resistance, but in contrast to *lsd* or *acd2,* do not display detectable lesions on their leaves. *cpr1* (constitutive expresser of PR genes) may be a type of *cim* mutant; however, because the presence of microscopic lesions on the leaves of *cpr1* has not been ruled out, *cpr1* might be a type of *lsd* mutant (Bowling et al., *Plant Cell* 6, 1845-1857 (1994), incorporated by reference herein in its entirety).

Mutants have also been isolated that are blocked in SAR signaling. *ndr1* (non-race-specific disease resistance) is a mutant that allows growth of both *Pseudomonas syringae* containing various avirulence genes and also normally avirulent isolates of *Peronospora parasitica* (Century et al., *Proc. Natl. Acad.Sci. USA* 92, 6597-6601 (1995), incorporated by reference herein in its entirety). Apparently this mutant is blocked early in SAR signaling. *npr1* (nonexpresser of PR genes) is a mutant that cannot induce expression of the SAR signaling pathway following INA treatment (Cao et al., *Plant Cell* 6, 1583-1592 (1994), incorporated by reference herein in its entirety). *eds* (enhanced disease susceptibility) mutants have been isolated based on their ability to support bacterial infection following

inoculation of a low bacterial concentration (Glazebrook et al., *Genetics* 143, 973-982 (1996), incorporated by reference herein in its entirety; Parker et al., *Plant Cell* 8, 2033-2046 (1996), incorporated by reference herein in its entirety). Certain *eds* mutants are phenotypically very similar to *npr1*, and, recently, *eds5* and *eds53* have been shown to be allelic to *npr1* (Glazebrook et al., 1996). *nim1* (noninducible immunity) is a mutant that supports *P. parasitica* (i.e., causal agent of downy mildew disease) growth following INA treatment (Delaney et al., 1995; International PCT Application WO 94/16077). Although *nim1* can accumulate SA following pathogen infection, it cannot induce SAR gene expression or disease resistance, suggesting that the mutation blocks the pathway downstream of SA. *nim1* is also impaired in its ability to respond to INA or BTH, suggesting that the block exists downstream of the action of these chemicals (Delaney et al., 1995; Lawton et al., 1996).

Recently, two allelic *Arabidopsis* genes have been isolated and characterized, mutants of which are responsible for the *nim1* and *npr1* phenotypes, respectively (Ryals *et al.*, *Plant Cell* 9, 425-439 (1997), incorporated by reference herein in its entirety; Cao *et al.*, *Cell* 88, 57-63 (1997), incorporated by reference herein in its entirety). The wild-type *NIM1* gene product is involved in the signal transduction cascade leading to both SAR and gene-for-gene disease resistance in *Arabidopsis* (Ryals *et al.*, 1997). Ryals *et al.*, 1997 also report the isolation of five additional alleles of *nim1* that show a range of phenotypes from weakly impaired in chemically induced PR-1 gene expression and fungal resistance to very strongly blocked. Transformation of the wild-type *NPR1* gene into *npr1* mutants not only complemented the mutations, restoring the responsiveness of SAR induction with respect to PR-gene expression and disease resistance, but also rendered the transgenic plants more resistant to infection by *P. syringae* in the absence of SAR induction (Cao *et al.*, 1997).

NF-κB/IκB Signal Transduction Pathways

NF-κB/IκB signaling pathways have been implicated in disease resistance responses in a range of organisms from *Drosophila* to mammals. In mammals, NF-κB/IκB signal transduction can be induced by a number of different stimuli including exposure of cells to lipopolysaccharide, tumor necrosis factor, interleukin 1 (IL-1), or virus infection (Baeuerle and Baltimore, *Cell* 87, 13-20 (1996); Baldwin, *Annu. Rev. Immunol.* 14, 649-681 (1996)). The activated pathway leads to the synthesis of a number of factors involved in inflammation and immune responses, such as IL-2, IL-6, IL-8 and granulocyte/macrophage-colony stimulating factor (deMartin et al., *Gene* 152, 253-255 (1995)). In transgenic mouse studies, the knock out of NF-κB/IκB signal transduction leads to a def ctive immune

response including enhanced susceptibility to bacterial and viral pathogens (Beg and Baltimore, *Science* 274, 782-784 (1996); Van Antwerp et al., *Science* 274, 787-789 (1996); Wang et al., *Science* 274, 784-787 (1996); Baeuerle and Baltimore (1996)). In Arabidopsis, SAR is functionally analogous to inflammation in that normal resistance processes are potentiated following SAR activation leading to enhanced disease resistance (Bi et al., 1995; Cao et al., 1994; Delaney et al., 1995; Delaney et al., 1994; Gaffney et al., 1993; Mauch-Mani and Slusarenko 1996; Delaney, 1997). Furthermore, inactivation of the pathway leads to enhanced susceptibility to bacterial, viral and fungal pathogens. Interestingly, SA has been reported to block NF-κB activation in mammalian cells (Kopp and Ghosh, *Science* 265, 956-959 (1994)), while SA activates signal transduction in Arabidopsis. Bacterial infection of Drosophila activates a signal transduction cascade leading to the synthesis of a number of antifungal proteins such as cercropin B, defensin, diptericin and drosomycin (Ip et al., *Cell* 75, 753-763 (1993); Lemaitre et al., *Cell* 86, 973-983 (1996)). This induction is dependent on the gene product of *dorsal* and *dif*, two NF-κB homologs, and is repressed by *cactus*, an IκB homolog, in the fly. Mutants that have decreased synthesis of the antifungal and antibacterial proteins have dramatically lowered resistance to infection.

Despite much research and the use of sophisticated and intensive crop-protection measures, including genetic transformation of plants, losses due to disease remain in the billions of dollars annually. Therefore, there is a continuing need to develop new crop protection measures based on the ever-increasing understanding of the genetic basis for disease resistance in plants.

The following definitions will assist in the understanding of the present invention.

Plant cell: the structural and physiological unit of plants, consisting of a protoplast and the cell wall. The term "plant cell" refers to any cell which is either part of or derived from a plant. Some examples of cells include differentiated cells that are part of a living plant; differentiated cells in culture; undifferentiated cells in culture; the cells of undifferentiated tissue such as callus or tumors; differentiated cells of seeds, embryos, propagules and pollen.

Plant tissue: a group of plant cells organized into a structural and functional unit. Any tissue of a plant in planta or in culture is included. This term includes, but is not limited to, whole plants, plant organs, plant seeds, tissue culture and any groups of plant cells organized into structural and/or functional units. The use of this term in conjunction with, or in the absence of, any specific type of plant tissue as listed above or otherwise embraced

by this definition is not intended to be exclusive of any other type of plant tissue.

Protoplast: a plant cell without a cell wall.

Descendant plant: a sexually or asexually derived future generation plant which includes, but is not limited to, progeny plants.

Transgenic plant: a plant having stably incorporated recombinant DNA in its genome.

Recombinant DNA: Any DNA molecule formed by joining DNA segments from different sources and produced using recombinant DNA technology.

Recombinant DNA technology: Technology which produces recombinant DNA *in vitro* and transfers the recombinant DNA into cells where it can be expressed or propagated (*See,* Concise Dictionary of Biomedicine and Molecular Biology, Ed. Juo, CRC Press, Boca Raton (1996)), for example, transfer of DNA into a protoplast(s) or cell(s) in various forms, including, for example, (1) naked DNA in circular, linear or supercoiled forms, (2) DNA contained in nucleosomes or chromosomes or nuclei or parts thereof, (3) DNA complexed or associated with other molecules, (4) DNA enclosed in liposomes, spheroplasts, cells or protoplasts or (5) DNA transferred from organisms other than the host organism (ex. *Agrobacterium tumefiaciens*). These and other various methods of introducing the recombinant DNA into cells are known in the art and can be used to produce the transgenic cells or transgenic plants of the present invention.

Recombinant DNA technology also includes the homologous recombination methods described in Treco *et al.*, WO 94/12650 and Treco *et al.*, WO 95/31560 which can be applied to increasing peroxidase activity in a monocot. Specifically, regulatory regions (ex. promoters) can be introduced into the plant genome to increase the expression of the endogenous peroxidase.

Also included as recombinant DNA technology is the insertion of a peroxidase coding sequence lacking selected expression signals into a monocot and assaying the transgenic monocot plant for increased expression of peroxidase due to endogenous control sequences in the monocot. This would result in an increase in copy number of peroxidase coding sequences within the plant.

The initial insertion of the recombinant DNA into the genome of the R^0 plant is not defined as being accomplished by traditional plant breeding methods but rather by technical methods as described herein. Following the initial insertion, transgenic descendants can be propagated using essentially traditional breeding methods.

Chimeric gene: A DNA molecule containing at least two heterologous parts, e.g., parts derived from pre-existing DNA sequences which are not associated in their pre-existing states, these sequences having been preferably generated using recombinant

DNA technology.

Expression cassette: a DNA molecule comprising a promoter and a terminator between which a coding sequence can be inserted.

Coding sequence: a DNA molecule which, when transcribed and translated, results in the formation of a polypeptide or protein.

Gene: a discrete chromosomal region comprising a regulatory DNA sequence responsible for the control of expression, i.e. transcription and translation, and of a coding sequence which is transcribed and translated to give a distinct polypeptide or protein.

The present invention describes the identification, isolation, and characterization of the *NIM1* gene, which encodes a protein involved in the signal transduction cascade responsive to biological and chemical inducers that leads to systemic acquired resistance in plants.

Hence, the present invention discloses an isolated DNA molecule (*NIM1* gene) that encodes a NIM1 protein involved in the signal transduction cascade leading to systemic acquired resistance in plants.

Within the scope of the present invention a DNA molecule is described that encodes the NIM1 protein hybridizing under the following conditions to clone BAC-04, ATCC Deposit No. 97543: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C. In an especially preferred embodiment, the *NIM1* gene is comprised within clone BAC-04, ATCC Deposit No. 97543.

Further described is a DNA molecule that encodes the NIM1 protein hybridizes under the following conditions to cosmid D7, ATCC Deposit No. 97736: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C. In an especially preferred embodiment, the *NIM1* gene is comprised within cosmid D7, ATCC Deposit No. 97736.

The *NIM1* gene described herein may be isolated from a dicotyledonous plant such as *Arabidopsis*, tobacco, cucumber, or tomato. Alternately, the *NIM1* gene may be isolated from a monocotyledonous plant such as maize, wheat, or barley.

Further described is an encoded NIM1 protein comprising the amino acid sequence set forth in SEQ ID NO:3. Further described is the *NIM1* gene coding sequence hybridizing under the following conditions to the coding sequence set forth in SEQ ID NO:2: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC

for 15 min. (X1) at 55°C. In an especially preferred embodiment, the *NIM1* gene coding sequence comprises the coding sequence set forth in SEQ ID NO:2.

The present invention also describes a chimeric gene comprising a promoter active in plants operatively linked to a *NIM1* gene coding sequence, a recombinant vector comprising such a chimeric gene, wherein the vector is capable of being stably transformed into a host, as well as a host stably transformed with such a vector. Preferably, the host is a plant such as one of the following agronomically important crops: rice, wheat, barley, rye, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane.

In an especially preferred embodiment, the NIM1 protein is expressed in a transformed plant at higher levels than in a wild type plant.

The present invention is also directed to a method of conferring a CIM phenotype to a plant by transforming the plant with a recombinant vector comprising a chimeric gene that itself comprises a promoter active in plants operatively linked to a *NIM1* gene coding sequence, wherein the encoded NIM1 protein is expressed in the transformed plant at higher levels than in a wild type plant.

Further, the present invention is directed to a method of activating systemic acquired resistance in a plant by transforming the plant with a recombinant vector comprising a chimeric gene that itself comprises a promoter active in plants operatively linked to a *NIM1* gene coding sequence, wherein the encoded NIM1 protein is expressed in the transformed plant at higher levels than in a wild type plant.

In addition, the present invention is directed to a method of conferring broad spectrum disease resistance to a plant by transforming the plant with a recombinant vector comprising a chimeric gene that itself comprises a promoter active in plants operatively linked to a *NIM1* gene coding sequence, wherein the encoded NIM1 protein is expressed in the transformed plant at higher levels than in a wild type plant.

Another aspect of the present invention exploits both the recognition that the SAR pathway in plants shows functional parallels to the NF-κB/IκB regulation scheme in mammals and flies, as well as the discovery that the *NIM1* gene product is a structural homologue of the mammalian signal transduction factor IκB subclass α. Mutations of IκB have been described that act as super-repressors or dominant-negatives of the NF-κB/IκB regulation scheme. The present invention encompasses alter d forms of wild-type *NIM1* gene (SEQ NO: 2) that act as dominant-negative regulators of the SAR signal transduction

pathway. These altered forms of *NIM1* confer the opposite phenotype in plants transformed therewith as the *nim1* mutant; plants i.e., plants transformed with altered forms of *NIM1* exhibit constitutive SAR gene expression and a CIM phenotype.

Also comprised by the present invention are DNA molecules that hybridize to a DNA molecule according to the invention as defined hereinbefore, but preferably to an oligonucleotide probe obtainable from said DNA molecule comprising a contiguous portion of the coding sequence for the said altered forms of *NIM1* at least 10 nucleotides in length, under moderately stringent conditions.

Factors that affect the stability of hybrids determine the stringency of the hybridization. One such factor is the melting temperature T_m which can be easily calculated according to the formula provided in DNA PROBES, George H. Keller and Mark M. Manak , Macmillan Publishers Ltd, 1993, Section one: Molecular Hybridization Technology; page 8 ff.

The preferred hybridization temperature is in the range of about 25°C below the calculated melting temperature T_m and preferably in the range of about 12-15°C below the calculated melting temperature T_m and in the case of oligonucleotides in the range of about 5-10°C below the melting temperature T_m.

In one embodiment of the present invention, the *NIM1* gene is altered so that the encoded product has alanines instead of serines in the amino acid positions corresponding to positions 55 and 59 of the wild-type *Arabidopsis* NIM1 amino acid sequence (SEQ ID NO:3). An example of a preferred embodiment of this altered form of the *NIM1* gene, which results in changes of these serine residues to alanine residues, is presented in SEQ ID NO:22. An exemplary dominant-negative form of the NIM1 protein with alanines instead of serines at amino acid positions 55 and 59 is shown in SEQ ID NO:23. The present invention also encompasses altered forms of alleles of *NIM1*, wherein the coding sequence of such an allele hybridizes under moderate stringent conditions to the coding sequence set forth in SEQ ID NO:22, especially preferred are the following conditions: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C. In these embodiments, alleles of *NIM1* hybridizing to SEQ ID NO:22 under the above conditions are altered so that the encoded product has alanines instead of serines in the amino acid positions that correspond to positions 55 and 59 of SEQ ID NO:22.

In another embodiment of the present invention, the *NIM1* gene is altered so that the encoded product has an N-terminal truncation, which removes lysine residues that may serve as potential ubiquitination sites in addition to the serines at amino acid positions corresponding to positions 55 and 59 of the wild-type protein. An example of a preferred

embodiment of this altered form of the *NIM1* gene, which encodes a gene product having an N-terminal deletion, is presented in SEQ ID NO:24. An exemplary dominant-negative form of the NIM1 protein with an N-terminal deletion is shown in SEQ ID NO:25. The present invention also encompasses altered forms of alleles of *NIM1*, wherein the coding sequence of such an allele hybridizes under moderate stringent conditions to the coding sequence set forth in SEQ ID NO:24; especially preferred are the following conditions: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C. In these embodiments, alleles of *NIM1* hybridizing to SEQ ID NO:24 under the above conditions are altered so that the encoded product has an N-terminal deletion that removes lysine residues that may serve as potential ubiquitination sites in addition to the serines at amino acid positions corresponding to positions 55 and 59 of the wild-type gene product.

In still another embodiment of the present invention, the *NIM1* gene is altered so that the encoded product has a C-terminal truncation, which is believed to result in enhanced intrinsic stability by blocking the constitutive phosporylation of serine and threonine residues in the C-terminus of the wild-type gene product. An example of a preferred embodiment of this altered form of the *NIM1* gene, which encodes a gene product having a C-terminal deletion, is presented in SEQ ID NO:26. An exemplary dominant-negative form of the NIM1 protein with a C-terminal deletion is shown in SEQ ID NO:27. The present invention also encompasses altered forms of alleles of *NIM1*, wherein the coding sequence of such an allele hybridizes under moderate stringent conditions to the coding sequence set forth in SEQ ID NO:26; especially preferred are the following conditions: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C. In these embodiments, alleles of *NIM1* hybridizing to SEQ ID NO:26 under the above conditions are altered so that the encoded product has a C-terminal deletion that removes serine and threonine residues.

In yet another embodiment of the present invention, the *NIM1* gene is altered so that the encoded product has both an N-terminal deletion and a C-terminal truncation, which provides the benefits of both the above-described embodiments of the invention.
A preferrred embodiment of the invention is an altered form of the NIM1 protein that has an N-terminal truncation of amino acids corresponding approximately to amino acid positions 1-125 of SEQ ID NO:2 and a C-terminal truncation of amino acids corresponding approximately to amino acid positions 522-593 of SEQ ID NO:3.

An example of a preferred embodiment of this altered form of the *NIM1* gene, which encodes a gene product having both an N-terminal and a C-terminal deletion, is presented in SEQ ID NO:28. An exemplary dominant-negative form of the NIM1 protein with a C-terminal deletion is shown in SEQ ID NO:29. The present invention also encompasses altered forms of alleles of *NIM1*, wherein the coding sequence of such an allele hybridizes under the moderate stringent conditions to the coding sequence set forth in SEQ ID NO:28; especially preferred are the following conditions: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C. In these embodiments, alleles of *NIM1* hybridizing to SEQ ID NO:28 under the above conditions are altered so that the encoded product has both an N-terminal deletion, which removes lysine residues that may serve as potential ubiquitination sites in addition to the serines at amino acid positions corresponding to positions 55 and 59 of the wild-type gene product, as well as a C-terminal deletion, which removes serine and threonine residues.

In even another embodiment of the present invention, the *NIM1* gene is altered so that the encoded product consists essentially of only the ankyrin domains of the wild-type gene product. Preferred is an isolated DNA molecule, wherein said altered form of the NIM1 protein consists essentially of ankyrin motifs corresponding approximately to amino acid positions 103-362 of SEQ ID NO:3. An example of a preferred embodiment of this altered form of the *NIM1* gene, which encodes the ankyrin domains, is presented in SEQ ID NO:30. An exemplary dominant-negative form of the NIM1 protein consists essentially of only the ankyrin domains is shown in SEQ ID NO:31. The present invention also encompasses altered forms of alleles of *NIM1*, wherein the coding sequence of such an allele hybridizes under the moderate stringent conditions to the coding sequence set forth in SEQ ID NO:30; especially preferred are the following conditions: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C. In these embodiments, alleles of *NIM1* hybridizing to SEQ ID NO:30 under the above conditions are altered so that the encoded product consists essentially of the ankyrin domains of the wild-type gene product.

Thus, the present invention concerns DNA molecules encoding altered forms of the *NIM1* gene, such as those described above and all DNA molecules hybridizing therewith using moderate stringent conditions.

The present invention also encompasses a chimeric gene comprising a promoter active in plants operatively linked to one of the above-described altered forms of the *NIM1* gene, a recombinant vector comprising such a chimeric gene, wherein the vector is capable

of being stably transformed into a host cell, as well as a host cell stably transformed with such a vector. Preferably, the host cell is a plant, plant cells and the descendants thereof from, for example, one of the following agronomically important crops: rice, wheat, barley, rye, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane.

The present invention is also directed to a method of conferring a CIM phenotype to a plant by transforming the plant with a recombinant vector comprising a chimeric gene that itself comprises a promoter active in plants operatively linked to one of the above-described altered forms of the *NIM1* gene, wherein the encoded dominant-negative form of the NIM1 protein is expressed in the transformed plant and confers a CIM phenotype to the plant.

Further, the present invention is directed to a method of activating systemic acquired resistance in a plant by transforming the plant with a recombinant vector comprising a chimeric gene that itself comprises a promoter active in plants operatively linked to one of the above-described altered forms of the *NIM1* gene, wherein the encoded dominant-negative form of the NIM1 protein is expressed in the transformed plant and activates systemic acquired resistance in the plant.

In addition, the present invention is directed to a method of conferring broad spectrum disease resistance to a plant by transforming the plant with a recombinant vector comprising a chimeric gene that itself comprises a promoter active in plants operatively linked to one of the above-described altered forms of the *NIM1* gene, wherein the encoded dominant-negative form of the NIM1 protein is expressed in the transformed plant and confers broad spectrum disease resistance to the plant.

In yet another aspect, the present invention is directed to a method of screening for a *NIM1* gene involved in the signal transduction cascade leading to systemic acquired resistance in a plant, comprising probing a genomic or cDNA library from said plant with a *NIM1* coding sequence that hybridizes under the following set of conditions to the coding sequence set forth in SEQ ID NO:2: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C.

Further subjects encompassed by the invention are:

An isolated DNA molecule according to the invention wherein said altered form of the NIM1 protein has alanines instead of serines in amino acid positions corresponding to positions

55 and 59 of SEQ ID NO:3, wherein said DNA molecule hybridizes under the following conditions to the nucleotide sequence set forth in SEQ ID NO:22: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C.

An isolated DNA molecule according to the invention wherein said altered form of the NIM1 protein has an N-terminal truncation of amino acids corresponding approximately to amino acid positions 1-125 of SEQ ID NO:3, wherein said DNA molecule hybridizes under the following conditions to the nucleotide sequence set forth in SEQ ID NO:24: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C.

An isolated DNA molecule according to the invention wherein said altered form of the NIM1 protein has a C-terminal truncation of amino acids corresponding approximately to amino acid positions 522-593 of SEQ ID NO:3, wherein said DNA molecule hybridizes under the following conditions to the nucleotide sequence set forth in SEQ ID NO:26: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C.

An isolated DNA molecule according to the invention, wherein said altered form of the NIM1 protein comprises the amino acid sequence shown in SEQ ID NO:28, wherein said DNA molecule hybridizes under the following conditions to the nucleotide sequence set forth in SEQ ID NO:28: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C.

An isolated DNA molecule according to the invention wherein said altered form of the NIM1 protein consists essentially of ankyrin motifs corresponding approximately to amino acid positions 103-362 of SEQ ID NO:3, wherein said DNA molecule hybridizes under the following conditions to the nucleotide sequence set forth in SEQ ID NO:30: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C.

An altered form of a *NIM1* gene according to the invention, which has been constructed by mutagenization.

Use of an isolated DNA molecule according to the invention to activate systemic acquired resistance in a plant cell, plant and the descendants thereof.

Use of an isolated DNA molecule according to the invention to confer a broad spectrum disease resistance to a plant cell, a plant and the descendants thereof.

Use of an isolated DNA molecule according to the invention to confer a CIM phenotype to a plant cell, a plant and the descendants thereof.

Use of resistant plants and the descendants thereof according to the invention to incorporate the disease resistant trait into plant lines through breeding.

Use of variants of the *NIM1* gene to confer disease resistance and activate SAR gene expression in plants transformed therewith.

A method of producing an altered form of a *NIM1* gene.

A method of producing transgenic descendants of a transgenic parent plant comprising an isolated DNA molecule encoding an altered form of a NIM1 protein according to the invention comprising transforming said parent plant with a recombinant vector molecule according to the invention and transferring the trait to the descendants of said transgenic parent plant involving known plant breeding techniques.

A method of producing a DNA molecule comprising a DNA portion containing a DNA portion encoding an altered form of a NIM1 protein

(a) preparing a nucleotide probe capable of specifically hybridizing to an altered form of a *NIM1* gene or mRNA, wherein said probe comprises a contiguous portion of the coding sequence for an altered form of a *NIM1* of at least 10 nucleotides length;

(b) probing for other altered forms of a *NIM1* coding sequence in populations of cloned genomic DNA fragments or cDNA fragments from a chosen organism using the nucleotide probe prepared according to step (a); and

(c) isolating and multiplying a DNA molecule comprising a DNA portion containing a DNA portion encoding an altered form of a NIM1 protein.

A method of isolating a DNA molecule comprising a DNA portion containing an altered form of a *NIM1* sequence comprising

(a) preparing a nucleotide probe capable of specifically hybridizing to an altered form of a *NIM1* gene or mRNA, wherein said probe comprises a contiguous portion of the coding sequence for an altered form of a NIM1 protein from a plant of at least 10 nucleotides length;

(b) probing for other altered forms of *NIM1* sequences in populations of cloned genomic DNA fragments or cDNA fragments from a chosen organism using the nucleotide probe prepared according to step (a); and

(c) isolating a DNA molecule comprising a DNA portion containing an altered form of a *NIM1* gene.

A method of producing transgenic plants that express higher-than-wild-type levels of the *NIM1* gene, or functional variants and mutants thereof.

A method of producing transgenic plants that express higher-than-wild-type levels of the *NIM1* gene, or functional variants and mutants thereof, wherein the expression of the *NIM1* gene is at a level which is at least two-fold above the expression level of the *NIM1* gene in wild-type plants.

A method of producing transgenic plants that express higher-than-wild-type levels of the *NIM1* gene, or functional variants and mutants thereof, wherein the expression of the *NIM1* gene is at a level which is at least ten-fold above the expression level of the *NIM1* gene in wild-type plants.

The *nim* Mutant Phenotype

The present invention relates to mutant plants, as well as genes isolated therefrom, which are defective in their normal response to pathogen infection in that they do not express genes associated with SAR. These mutants are referred to as *nim* mutants (for non-inducible immunity) and are "universal disease susceptible" (UDS) by virtue of their being susceptible to many strains and pathotypes of pathogens of the host plant and also to pathogens that do not normally infect the host plant, but that normally infect other hosts. Such mutants can be selected by treating seeds or other biological material with mutagenic agents and then selecting descendant plants for the UDS phenotype by treating

descendant plants with known chemical inducers (e.g. INA) of SAR and then infecting the plants with a known pathogen. Non-inducible mutants develop severe disease symptoms under these circumstances, whereas wild type plants are induced by the chemical compound to systemic acquired resistance. *nim* mutants can be equally selected from mutant populations generated by chemical and irradiation mutagenesis, as well as from populations generated by T-DNA insertion and transposon-induced mutagenesis. Techniques of generating mutant plant lines are well known in the art.

nim mutants provide useful indicators of the evaluation of disease pressure in field pathogenesis tests where the natural resistance phenotype of so-called wild type (i.e. non-mutant) plants may vary and therefore not provide a reliable standard of susceptibility. Furthermore, *nim* plants have additional utility for the testing of candidate disease resistance transgenes. Using a *nim* stock line as a recipient for transgenes, the contribution of the transgene to disease resistance is directly assessable over a base level of susceptibility. Furthermore, the *nim* plants are useful as a tool in the understanding of plant-pathogen interactions. *nim* host plants do not mount a systemic response to pathogen attack, and the unabated development of the pathogen is an ideal system in which to study its biological interaction with the host.

As *nim* host plants may also be susceptible to pathogens outside of the host-range they normally fall, these plants also have significant utility in the molecular, genetic, and biological study of host-pathogen interactions. Furthermore, the UDS phenotype of *nim* plants also renders them of utility for fungicide screening. *nim* mutants selected in a particular host have considerable utility for the screening of fungicides using that host and pathogens of the host. The advantage lies in the UDS phenotype of the mutant, which circumvents the problems encountered by hosts being differentially susceptible to different pathogens and pathotypes, or even resistant to some pathogens or pathotypes.

nim mutants have further utility for the screening of fungicides against a range of pathogens and pathotypes using a heterologous host, i.e. a host that may not normally be within the host species range of a particular pathogen. Thus, the susceptibility of *nim* mutants of Arabidopsis to pathogens of other species (e.g. crop plant species) facilitates efficacious fungicide screening procedures for compounds against important pathogens of crop plants.

The *Arabidopsis thaliana nim1* Mutant

An *Arabidopsis thaliana* mutant called *nim1* (noninducible immunity) that supports *P. parasitica* (i.e., causal agent of downy mildew disease) growth following INA treatment is

described in Delaney et al., 1995. Although *nim1* can accumulate SA following pathogen infection, neither SAR gene expression nor disease resistance can be induced, suggesting that the mutation blocks the pathway downstream of SA. *nim1* is also impaired in its ability to respond to INA or BTH, suggesting that the block exists downstream of the action of these chemicals (Delaney et al., 1995; Lawton et al., 1996). This first Arabidopsis *nim1* mutant (herein designated *nim1-1*) was isolated from 80,000 plants of a T-DNA tagged Arabidopsis ecotype Issilewskija (Ws-0) population by spraying two week old plants with 0.33 mM INA followed by inoculation with *P. parasitica* (Delaney et al., 1995). Plants that supported fungal growth after INA treatment were selected as putative mutants. Five additional mutants (herein designated *nim1-2, nim1-3, nim1-4, nim1-5,* and *nim1-6*) were isolated from 280,000 M_2 plants from an ethyl methanesulfonate (EMS)-mutagenized Ws-0 population.

To determine whether the mutants were dominant or recessive, Ws-0 plants were used as pollen donors to cross to each of these mutants. The F_1 plants were then scored for their ability to support fungal growth following INA treatment. As shown in Table 3 of the Examples, all *nim1-1, nim1-2, nim1-3, nim1-4,* and *nim1-6* F_1 plants were phenotypically wild type, indicating a recessive mutation in each line. *nim1-5* showed the *nim* phenotype in all 35 F_1 plants, indicating that this particular mutant is dominant. For verification, the reciprocal cross was carried out using *nim1-5* as the pollen donor to fertilize Ws-0 plants. In this case, all 18 F_1 plants were phenotypically *nim,* confirming the dominance of the *nim1-5* mutation.

To determine whether the *nim1-2* through *nim1-6* mutations were allelic to the previously characterized *nim1-1* mutation, pollen from *nim1-1* was used to fertilize *nim1-2* through *nim1-6.* Because *nim1-1* carried resistance to kanamycin, F_1 descendants were identified by antibiotic resistance. In all cases, the kanamycin-resistant F_1 plants were *nim,* indicating they were all allelic to the *nim1-1* mutant. Because the *nim1-5* mutant is dominant and apparently homozygous for the mutation, it was necessary to analyze *nim1-1* complementation in the F_2 generation. If *nim1-1* and *nim1-5* were allelic, then the expectation would be that all F_2 plants have a *nim* phenotype. If not, then 13 of 16 F_2 plants would have been expected to have a *nim* phenotype. Of 94 plants, 88 clearly supported fungal growth following INA treatment. Six plants showed an associated phenotype of black specks on the leaves reminiscent of a lesion mimic phenotype and supported little fungal growth following INA treatment. Because *nim1-5* carries a point mutation in the *NIM1* gene (*infra*), it is considered to be a *nim1* allele.

To determine the relative strength of the different *nim1* alleles, each mutant was analyzed for the growth of *P. parasitica* under normal growth conditions and following

pretreatment with either SA, INA, or BTH. As shown in Table 1, during normal growth, *nim1-1*, *nim1-2*, *nim1-3*, *nim1-4*, and *nim1-6* all supported approximately the same rate of fungal growth, which was somewhat faster than the Ws-0 control. The exception was the *nim1-5* plants, in which fungal growth was delayed by several days relative to both the other *nim1* mutants and the Ws-0 control, but eventually all of the *nim1-5* plants succumbed to the fungus. Following SA treatment, the mutants could be grouped into three classes: *nim1-4* and *nim1-6* showed a relatively rapid fungal growth; *nim1-1*, *nim1-2*, *nim1-3* plants exhibited a somewhat slower rate of fungal growth; and fungal growth in *nim1-5* plants was even slower than in the untreated Ws-0 controls. Following either INA or BTH treatment, the mutants also seemed to fall into three classes where *nim1-4* was the most severely compromised in its ability to restrict fungal growth following chemical treatment; *nim1-1*, *nim1-2*, *nim1-3*, and *nim1-6* were all moderately compromised; and *nim1-5* was only slightly compromised. In these experiments, Ws-0 did not support fungal growth following INA or BTH treatment. Thus, with respect to inhibition of fungal growth following chemical treatment, the mutants fall into three classes with *nim1-4* being the most severely compromised, *nim1-1*, *nim1-2*, *nim1-3* and *nim1-6* showing an intermediate inhibition of fungus and *nim1-5* with only slightly impaired fungal resistance.

The accumulation of *PR-1* mRNA was also used as a criterion to characterize the different *nim1* alleles. RNA was extracted from plants 3 days after either water or chemical treatment, or 14 days after inoculation with a compatible fungus (*P. parasitica* isolate Emwa). The RNA gel blot in Figure 3 shows that *PR-1* mRNA accumulated to high levels following treatment of wild-type plants with SA, INA, or BTH or infection by *P. parasitica*. In the *nim1-1*, *nim1-2*, and *nim1-3* plants, *PR-1* mRNA accumulation was dramatically reduced relative to the wild type following chemical treatment. *PR-1* mRNA was also reduced following *P. parasitica* infection, but there was still some accumulation in these mutants. In the *nim1-4* and *nim1-6* plants, *PR-1* mRNA accumulation was more dramatically reduced than in the other alleles following chemical treatment (evident in longer exposures) and significantly less *PR-1* mRNA accumulated following *P. parasitica* infection, supporting the idea that these could be particularly strong *nim1* alleles. Interestingly, *PR-1* mRNA accumulation was elevated in the *nim1-5* mutant, but only mildly induced following chemical treatment or *P. parasitica* infection. Based on both *PR-1* mRNA accumulation and fungal infection, the mutants fall into three classes: severely compromised alleles (*nim1-4* and *nim1-6*); moderately compromised alleles (*nim1-1*, *nim1-2*, and *nim1-3*); and a weakly compromised allele (*nim1-5*).

Fine Structure Mapping of the *nim1* Mutation

To determine a rough map position for *NIM1*, 74 F_2 *nim* phenotype plants from a cross between *nim1-1* (Ws-0) and Landsberg *erecta* (L*er*) were identified for their susceptibility to *P. parasitica* and lack of accumulation of *PR-1* mRNA following INA treatment. After testing a number of simple sequence length polymorphism (SSLP) markers (Bell and Ecker 1994), *nim1* was found to lie about 8.2 centimorgans (cM) from nga128 and 8.2 cM from nga111 on the lower arm of chromosome 1. In subsequent analysis, *nim1-1* was found to lie between nga111 and about 4 cM from the SSLP marker ATHGENEA.

For fine structure mapping, 1138 *nim* plants from an F_2 population derived from a cross between *nim1-1* and L*er*DP23 were identified based on both their inability to accumulate *PR-1* mRNA and their ability to support fungal growth following INA treatment. DNA was extracted from these plants and scored for zygosity at both ATHGENEA and nga111. As shown in Figures 5A-5D, 93 recombinant chromosomes were identified between ATHGENEA and *nim1*, giving a genetic distance of approximately 4.1 cM (93 of 2276), and 239 recombinant chromosomes were identified between nga111 and *nim1*, indicating a genetic distance of about 10.5 cM (239 of 2276). Informative recombinants in the ATHGENEA to nga111 interval were further analyzed using amplified fragment length polymorphism (AFLP) analysis (Vos et al., 1995).

Initially, 10 AFLP markers between ATHGENEA and nga111 were identified and these were used to construct a low resolution map of the region (Figure 5A). The AFLP markers W84.2 (1 cM from *nim1*) and W85.1 (0.6 cM from *nim1*) were used to isolate yeast artificial chromosome (YAC) clones from the CIC (for Centre d'Etude du Polymorphisme Humain, INRA and CNRS) library (Creusot et al., 1995). Two YAC clones, CIC12H07 and CIC12F04, were identified with W84.2 and two YAC clones CIC7E03 and CIC10G07 (data not shown) were identified with the W85.1 marker. However, it was determined that there was a gap between the two sets of flanking YAC clones. From this point, bacterial artificial chromosome (BAC) and P1 clones that overlapped CIC12H07 and CIC12F04 were isolated and mapped, and three sequential walking steps were then carried out extending the BAC/P1 contig toward *NIM1* (Liu et al., 1995; Chio et al., 1995). At various times during the walk, new AFLPs were developed that were specific for BAC or P1 clones, and these were used to determine whether the *NIM1* gene had been crossed. It was determined that *NIM1* had been crossed when BAC and P1 clones were isolated that gave rise to both AFLP markers L84.6a and L84.8. The AFLP marker L84.6a found on P1 clones P1-18, P1-17, and P1-21 identified three recombinants and L84.8 found on P1 clones P1-20, P1-22, P1-23, and P1-24 and BAC clones, BAC-04, BAC-05, and BAC-06 identified one

recombinant. Because these clones overlap to form a large contig (>100 kb), and include AFLP markers that flank *nim1*, the gene was located on the contig. The BAC and P1 clones that comprised the contig were used to generate eight additional AFLP markers, which showed that *nim1* was located between L84.Y1 and L84.8, representing a gap of about 0.09 cM.

A cosmid library was constructed in the *Agrobacterium*-compatible T-DNA cosmid vector pCLD04541 using DNA from BAC-06, BAC-04, and P1-18. A cosmid contig was developed using AFLP markers derived from these clones. Physical mapping showed that the physical distance between L84.Y1 and L84.8 was greater than 90 kb, giving a genetic to physical distance of roughly 1 megabase per cM. To facilitate the later identification of the *NIM1* gene, the DNA sequence of BAC-04 was determined.

Isolation of the *NIM1* Gene

To identify which cosmids contained the *NIM1* gene, the 12 cosmids listed in Table 4 of the Examples were transformed into *nim1-1*, and transformants were evaluated for their ability to complement the mutant phenotype. Cosmids D5, E1, and D7 were all found to complement *nim1-1*, as determined by the ability of the transformants to accumulate *PR-1* mRNA following INA treatment. The ends of these cosmids were sequenced and found to be located on the DNA sequence of BAC-04. There were 9,918 base pairs in the DNA region shared by D7 and D5 that contained the *NIM1* gene. As shown in Figure 5D, four putative gene regions were identified in this 10-kb sequence. Region 1 could potentially encode a protein of 19,105 D, region 3 could encode a protein of 44,554 D, and region 4 could encode a protein of 52,797 D. Region 2 had four open reading frames of various sizes located close together, suggesting a gene with three introns. Analysis using the NetPlantGene program (Hebsgaard et al., 1996) indicated a high probability that the open reading frames could be spliced together to form a large open reading frame encoding a protein of 66,039 D.

To ascertain which gene region contained the *NIM1* gene, gel blots containing RNA isolated from leaf tissue of Ws-0 and the different *nim1* mutants following either water or chemical treatment were probed with DNA derived from each of the four gene regions. In these experiments, care was taken to label probes to high specific activity and autoradiographs were exposed for more than 1 week. In our past experience, these conditions would identify RNA at concentrations of about one copy per cell. The only gene region that produced detectable RNA was gene region 2. As shown in Figure 7, the mRNA identified by the gene region 2 probe was induced by BTH treatment of wild-type plants, but

not in any of the mutants. Furthermore, RNA accumulation was elevated in all of the plants following *P. parasitica* infection, indicating that this particular gene is induced following pathogen infection.

To further establish the gene region encoding *NIM1*, the DNA sequence from each of the four gene regions was determined for each of the *nim1* alleles and compared with the corresponding gene region from Ws-0. No mutations were detected between Ws-0 and the mutant alleles in either gene regions 3 or 4 and only a single change was found in gene region 1 in the *nim1-6* mutant. However, a single base pair mutation was found in each of the alleles relative to Ws-0 for region 2. The DNA sequence of gene region 2 is shown in Figure 6. As shown in Table 5 and Figure 6, in *nim1-1*, a single adenosine is inserted at position 3579 that causes a frameshift resulting in a change in seven amino acids and a deletion of 349 amino acids. In *nim1-2*, there is a cytidine-to-thymidine transition at position 3763 that changes a histidine to a tyrosine. In *nim1-3*, a single adenosine is deleted at position 3301 causing a frameshift that altered 10 amino acids and deleted 412 from the predicted protein. Interestingly, both *nim1-4* and *nim1-5* have a guanosine-to-adenosine transition at position 4160 changing an arginine to a lysine, and in *nim1-6*, there is a cytosine-to-thymidine transition resulting in a stop codon causing the deletion of 255 amino acids from the predicted protein. Although the mutation in *nim1-4* and *nim1-5* alters the consensus donor splice site for the mRNA, RT-PCR analysis indicates that this mutation does not lead to an alteration of mRNA splicing (data not shown).

NIM1 Homologues

The gene region 2 DNA sequence was used in a Blast search (Altschul et al., 1990) and identified an exact match with the Arabidopsis expressed sequence tag (EST) T22612 and significant matches to the rice ESTs S2556, S2861, S3060 and S3481 (see Figure 8). A DNA probe covering base pairs 2081 to 3266 was used to screen an Arabidopsis cDNA library, and 14 clones were isolated that correspond to gene region 2. From the cDNA sequence, we could confirm the placement of the exon/intron borders shown in Figure 6. Rapid amplification of cDNA ends by polymerase chain reaction (RACE) was carried out using RNA from INA-treated Ws-0 plants and the likely transcriptional start site was determined to be the A at position 2588 in Figure 6.

Using the *NIM1* cDNA as a probe, homologs of Arabidopsis *NIM1* can be identified and isolated through screening genomic or cDNA libraries from different plants such as, but not limited to following crop plants: rice, wheat, barley, rye, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish,

spinach, asparagus, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane. Standard techniques for accomplishing this include hybridization screening of plated DNA libraries (either plaques or colonies; see, *e.g.* Sambrook *et al.*, Molecular Cloning , eds., Cold Spring Harbor Laboratory Press. (1989)) and amplification by PCR using oligonucleotide primers (see, *e.g.* Innis *et al.*, PCR Protocols, a Guide to Methods and Applications eds., Academic Press (1990)). Homologues identified are genetically engineered into the expression vectors listed below and transformed into the above listed crops. Transformants are evaluated for enhanced disease resistance using relevant pathogens of the crop plant being tested.

For example, *NIM1* homologs in the genomes of cucumber, tomato, tobacco, maize, wheat and barley have been detected by DNA blot analysis. Genomic DNA was isolated from cucumber, tomato, tobacco, maize, wheat and barley, restriction digested with the enzymes BamHI, HindIII, XbaI, or SalI, electrophoretically separated on 0.8% agarose gels and transferred to nylon membrane by capillary blotting. Following UV-crosslinking to affix the DNA, the membrane was hybridized under low stringency conditions [(1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride) at 55°C for 18-24h] with ^{32}P-radiolabelled *Arabidopsis thaliana* NIM1 cDNA. Following hybridization the blots were washed under low stringency conditions [6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C; 1XSSC is 0.15M NaCl, 15mM Na-citrate (pH7.0)] and exposed to X-ray film to visualize bands that correspond to *NIM1*.

In addition, expressed sequence tags (EST) identified with similarity to the *NIM1* gene such as the rice EST's described above can also be used to isolate homologues. The rice EST's may be especially useful for isolation of *NIM1* homologues from other monocots.

Homologues may also be obtained by PCR. In this method, comparisons are made between known homologues (e.g., rice and Arabidopsis). Regions of high amino acid and DNA similarity or identity are then used to make PCR primers. Once a suitable region is identified, primers for that region are made with a diversity of substitutions in the 3rd codon position. The PCR reaction is performed from cDNA or genomic DNA under a variety of standard conditions. When a band is apparent, it is cloned and/or sequences to determine if it is a *NIM1* homologue.

Overexpression of *NIM1* Confers Disease Resistance In Plants

The present invention also concerns the production of transgenic plants that express higher-than-wild-type levels of the *NIM1* gene, or functional variants and mutants thereof, and thereby have broad spectrum disease resistance. In a preferred embodiment of the invention, the expression of the *NIM1* gene is at a level which is at least two-fold above the expression level of the *NIM1* gene in wild-type plants and is preferably tenfold above the wild-type expression level. Overexpression of the *NIM1* gene mimics the effects of inducer compounds in that it gives rise to plants with a constitutive immunity (CIM) phenotype.

Several methods are described for producing plants that overexpress the *NIM1* gene and thereby have a CIM phenotype. A first method is selecting transformed plants that have high-level expression of *NIM1* and therefore a CIM phenotype due to insertion site effect. Table 6 shows the results of testing of various transformants for resistance to fungal infection. As can be seen from this table, a number of transformants showed less than normal fungal growth and several showed no visible fungal growth at all. RNA was prepared from collected samples and analyzed as described in Delaney et al, 1995. Blots were hybridized to the *Arabidopsis* gene probe PR-1 (Uknes et al, 1992). Three lines showed early induction of PR-1 gene expression in that PR-1 mRNA was evident by 24 or 48 hours following fungal treatment. These three lines also demonstrated resistance to fungal infection.

In addition, methods are described for constructing plant transformation vectors comprising a constitutive plant-active promoter, such as the CaMV 35S promoter, operatively linked to a coding region that encodes an active NIM1 protein. High levels of the active NIM1 protein produce the same disease-resistance effect as chemical induction with inducing chemicals such as BTH, INA, and SA.

The *NIM1* Gene Is A Homolog Of IκBα

The *NIM1* gene is a key component of the systemic acquired resistance (SAR) pathway in plants (Ryals *et al.*,1996). The *NIM1* gene is associated with the activation of SAR by chemical and biological inducers and, in conjunction with such inducers, is required for SAR and SAR gene expression. The location of the *NIM1* gene was determined by molecular biological analysis of the genome of mutant plants known to carry the mutant *nim1* gene, which gives the host plants extreme sensitivity to a wide variety of pathogens and renders them unable to respond to pathogens and chemical inducers of SAR. The

wildtype *NIM1* gene of *Arapidopsis* has been mapped and sequenced (SEQ ID NO:2). The wild-type *NIM1* gene product (SEQ ID NO:3) is involved in the signal transduction cascade leading to both SAR and gene-for-gene disease resistance in *Arabidopsis* (Ryals *et al.*, 1997). Recombinant overexpression of the wild-type form of *NIM1* gives rise to plants with a constitutive immunity (CIM) phenotype and therefore confers disease resistance in transgenic plants. Increased levels of the active NIM1 protein produce the same disease-resistance effect as chemical induction with inducing chemicals such as BTH, INA, and SA.

The sequence of the *NIM1* gene (SEQ ID NO:2) was used in BLAST searches, and matches were identified based on homology of one rather highly conserved domain in the NIM1 gene sequence to ankyrin domains found in a number of proteins such as spectrins, ankyrins, NF-κB and IκB (Michaely and Bennett, *Trends Cell Biol.* 2, 127-129 (1992)). Beyond the ankyrin motif, however, conventional computer analysis did not detect other strong homologies, including homology to IκBα. Despite the failings of the computer programs, pair-wise visual inspections between the NIM1 protein (SEQ ID NO:3) and 70 known ankyrin-containing proteins were carried out, and striking similarities were found to members of the IκBα class of transcription regulators (Baeuerle and Baltimore 1996; Baldwin 1996). As shown in Figure 9, the NIM1 protein (SEQ ID NO:3) shares significant homology with IκBα proteins from mouse, rat, and pig (SEQ ID NOs: 18, 19, and 20, respectively).

NIM1 contains several important structural domains of IκBα throughout the entire length of the protein, including ankyrin domains (indicated by the dashed underscoring in Figure 9), 2 amino-terminal serines (amino acids 55 and 59 of NIM1) , a pair of lysines (amino acids 99 and 100 in NIM1) and an acidic C-terminus. Overall, NIM1 and IκBα share identity at 30% of the residues and conservative replacements at 50% of the residues. Thus, there is homology between IκBα and NIM1 throughout the proteins, with an overall similarity of 80%.

One way in which IκBα protein functions in signal transduction is by binding to the cytosolic transcription factor NF-κB and preventing it from entering the nucleus and altering transcription of target genes (Baeuerle and Baltimore, 1996; Baldwin, 1996). The target genes of NF-κB regulate (activate or inhibit) several cellular processes, including antiviral, antimicrobial and cell death responses (Baeuerle and Baltimore, 1996). When the signal transduction pathway is activated, IκBα is phosphorylated at two serine residues (amino acids 32 and 36 of Mouse IκBα). This programs ubiquitination at a double lysine (amino acids 21 and 22 of Mouse IκBα). Following ubiquitination, the NF-κB/IκB complex is routed through the proteosome where IκBα is degraded and NF-κB is released to the nucleus.

The phosphorylated serine residues important in IκBα function are conserved in NIM1 within a large contiguous block of conserved sequence from amino acids 35 to 84 (Figure 9). In contrast to IκBα, where the double lysine is located about 15 amino acids toward the N-terminus of the protein, in NIM1 a double lysine is located about 40 amino acids toward the C-terminal end. Furthermore, a high degree of homology exists between NIM1 and IκBα in the serine/threonine rich carboxy terminal region which has been shown to be important in basal turnover rate (Sun *et al., Mol. Cell. Biol.* 16, 1058-1065 (1996)). According to the present invention based on the analysis of structural homology and the presence of elements known to be important for IκBα function, NIM1 is expected to function like the IκBα, having analogous effects on plant gene regulation.

Plants containing the wild-type *NIM1* gene when treated with inducer chemicals are predicted to have more *NIM1* gene product (IκB homolog) or less phosphorylation of the *NIM1* gene product (IκB homolog). In accordance with this model, the result is that the plant NF-κB homolog is kept out of the nucleus, and SAR gene expression and resistance responses are allowed to occur. In the *nim1* mutant plants a non-functional *NIM1* gene product is present. Therefore, in accordance with this model, the NF-κB homolog is free to go to the nucleus and repress resistance and SAR gene expression.

Consistent with this idea, animal cells treated with salicylic acid show increased stability/abundance of IκB and a reduction of active NF-κB in the nucleus (Kopp and Ghosh, 1994). Mutations of IκB are known that act as super-repressors or dominant-negatives (Britta-Mareen Traenckner et al., *EMBO* 14: 2876-2883 (1995); Brown et al., *Science* 267: 1485-1488 (1996); Brockman et al., *Molecular and Cellular Biology* 15: 2809-2818 (1995); Wang et al., *Science* 274: 784-787 (1996)). These mutant forms of IκB bind to NF-κB but are not phosphorylated or ubiquitinated and therefore are not degraded. NF-κB remains bound to the IκB and cannot move into the nucleus.

Altered Forms Of The *NIM1* Gene

In view of the above, the present invention encompasses altered forms of *NIM1* that act as dominant-negative regulators of the SAR signal transduction pathway. Plants transformed with these dominant negative forms of *NIM1* have the opposite phenotype as *nim1* mutant plants in that the plants transformed with altered forms of *NIM1* exhibit constitutive SAR gene expression and therefore a CIM phenotype. Because of the position the *NIM1* gene holds in the SAR signal transduction pathway, it is expected that a number of alterations to the gene, beyond those specifically disclosed herein, will result in constitutive expression of SAR genes and, therefore, a CIM phenotype.

Phosphorylation of serine residues in human IκBα is required for stimulus activated degradation of IκBα thereby activating NF-κB. Mutagenesis of the serine residues (S32 and S36) in human IκBα to alanine residues inhibits stimulus-induced phosphorylation, thus blocking IκBα proteosome-mediated degradation (Traenckner *et al.*, 1995; Brown *et al.*, 1996; Brockman *et al.*, 1995; Wang *et al.*, 1996). This altered form of IκBα can function as a dominant-negative form by retaining NF-κB in the cytoplasm thereby blocking downstream signaling events. Based on the amino acid sequence comparison between NIM1 and IκB shown in Figure 9, serines 55 (S55) and 59 (S59) in NIM1 (SEQ ID NO:3) are homologous to S32 and S36 in human IκBα. To construct dominant-negative forms of NIM1, the serines at amino acid positions 55 and 59 are mutagenized to alanine residues. Thus, in a preferred embodiment of the present invention, the *NIM1* gene is altered so that the encoded product has alanines instead of serines in the amino acid positions corresponding to positions 55 and 59 of the *Arabidopsis* NIM1 amino acid sequence. The present invention also encompasses disease-resistant transgenic plants transformed with such an altered form of the *NIM1* gene, as well as methods of using this altered form of the *NIM1* gene to confer disease resistance and activate SAR gene expression in plants transformed therewith.

Deletion of amino acids 1-36 (Brockman *et al.*, 1995; Sun *et al.*, 1996) or 1-72 (Sun *et al.*, 1996) of human IkBa, which includes ubiquination lysine residues K21 and K22 as well as phosphorylation sites S32 and S36, results in a dominant-negative IkBa phenotype in transfected human cell cultures. An N-terminal deletion of the first 125 amino acids of the *NIM1* gene product will remove eight lysine residues which could serve as ubiquination sites as well as the putative phosphorylation sites at S55 and S59 discussed above. Thus, in a preferred embodiment of the present invention, the *NIM1* gene is altered so that the encoded product is missing approximately the first 125 amino acids compared to the native *Arabidopsis* NIM1 amino acid sequence. The present invention also encompasses disease-resistant transgenic plants transformed with such an altered form of the *NIM1* gene, as well as methods of using this altered form of the *NIM1* gene to confer disease resistance and activate SAR gene expression in plants transformed therewith.

Deletion of amino acids 261-317 of human IkBa may result in enhanced intrinsic stability by blocking constitutive phosphorylation of serine and threonine residues in the C-terminus. This altered form of IκBα is expected to function as a dominant-negative form. A region rich in serine and threonine is present at amino acids 522-593 in the C-terminus of NIM1. Thus, in a preferred embodiment of the present invention, the *NIM1* gene is altered so that the encoded product is missing approximately its C-terminal portion, including amino acides 522-593, compared to the native *Arabidopsis* NIM1 amino acid sequence. The

present invention also encompasses disease-resistant transgenic plants transformed with such an altered form of the *NIM1* gene, as well as methods of using this altered form of the *NIM1* gene to confer disease resistance and activate SAR gene expression in plants transformed therewith.

In another embodiment of the present invention, altered forms of the *NIM1* gene product are produced as a result of C-terminal and N-terminal segment deletions or chimeras. In yet another embodiment of the present invention, constructs comprising the ankyrin domains from the *NIM1* gene are provided. The present invention encompasses disease-resistant transgenic plants transformed with such *NIM1* chimera or ankyrin constructs, as well as methods of using these variants of the *NIM1* gene to confer disease resistance and activate SAR gene expression in plants transformed therewith.

The present invention concerns DNA molecules encoding altered forms of the *NIM1* gene such as those described above, expression vectors containing such DNA molecules, and plants and plant cells transformed therewith. The invention also concerns methods of activating SAR in plants and conferring to plants a CIM phenotype and broad spectrum disease resistance by transforming the plants with DNA molecules encoding altered forms of the *NIM1* gene product. The present invention additionally concerns plants transformed with an altered form of the *NIM1* gene.

Disease Resistance

The overexpression of the wild-type *NIM1* gene in plants and the expression of altered forms of the *NIM1* gene in plants results in immunity to a wide array of plant pathogens, which include, but are not limited to viruses or viroids, e.g. tobacco or cucumber mosaic virus, ringspot virus or necrosis virus, pelargonium leaf curl virus, red clover mottle virus, tomato bushy stunt virus, and like viruses; fungi, e.g. *Phythophthora parasitica* and *Peronospora tabacina*; bacteria, e.g. *Pseudomonas syringae* and *Pseudomonas tabaci*; insects such as aphids, e.g. *Myzus persicae*; and lepidoptera, e.g., *Heliothus spp.*; and nematodes, e.g., *Meloidogyne incognita*. The vectors and methods of the invention are useful against a number of disease organisms including but not limited to downy mildews such as *Sclerophthora macrospora*, *Sclerophthora rayissiae*, *Sclerospora graminicola*, *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Peronosclerospora sacchari* and *Peronosclerospora maydis*; rusts such as *Puccinia sorphi*, *Puccinia polysora* and *Physopella zeae*; other fungi such as *Cercospora zeae-maydis*, *Colletotrichum graminicola*, *Fusarium monoliforme*, *Gibberella zeae*, *Exserohilum turcicum*, *Kabatiellu zeae*, *Erysiphe graminis*, *Septoria* and *Bipolaris maydis*; and bacteria such as *Erwinia stewartii*.

The methods of the present invention can be utilized to confer disease resistance to a wide variety of plants, including gymnosperms, monocots, and dicots. Although disease resistance can be conferred upon any plants falling within these broad classes, it is particularly useful in agronomically important crop plants, such as rice, wheat, barley, rye, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane. Transformed cells can be regenerated into whole plants such that the gene imparts disease resistance to the intact transgenic plants. The expression system can be modified so that the disease resistance gene is continuously or constitutively expressed.

Recombinant DNA Technology

The *NIM1* DNA molecule or gene fragment conferring disease resistance to plants by allowing induction of SAR gene expression or the altered form of the *NIM1* gene conferring disease resistance to plants by enhancing SAR gene expression can be incorporated in plant or bacterial cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule comprised within SEQ ID NO:1 or a functional variant thereof or a molecule encoding one of the altered forms of *NIM1* described above into an expression system to which the DNA molecule is heterologous (i.e., not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences. A large number of vector systems known in the art can be used, such as plasmids, bacteriophage viruses and other modified viruses. Suitable vectors include, but are not limited to, viral vectors such as lambda vector systems λgtl1, λgtl0 and Charon 4; plasmid vectors such as pBI121, pBR322, pACYC177, pACYC184, pAR series, pKK223-3, pUC8, pUC9, pUC18, pUC19, pLG339, pRK290, pKC37, pKC101, pCDNAII; and other similar systems. The *NIM1* coding sequence and the altered *NIM1* coding sequences described herein can be cloned into the vector using standard cloning procedures in the art, as described by Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory, Cold Spring Harbor, New York (1982).

In order to obtain efficient expression of the gene or gene fragment of the present invention, a promoter that will result in a sufficient expression level or constitutive

expression must be present in the expression vector. RNA polymerase normally binds to the promoter and initiates transcription of a gene. Promoters vary in their strength, i.e., ability to promote transcription. Depending upon the host cell system utilized, any one of a number of suitable promoters can be used. The components of the expression cassette may be modified to increase expression. For example, truncated sequences, nucleotide substitutions or other modifications may be employed. Plant cells transformed with such modified expression systems, then, exhibit overexpression or constitutive expression of genes necessary for activation of SAR.

A. Construction of Plant Transformation Vectors

Numerous transformation vectors are available for plant transformation, and the genes of this invention can be used in conjunction with any such vectors. The selection of vector will depend upon the preferred transformation technique and the target species for transformation. For certain target species, different antibiotic or herbicide selection markers may be preferred. Selection markers used routinely in transformation include the *nptII* gene which confers resistance to kanamycin and related antibiotics (Messing & Vierra. Gene 19: 259-268 (1982); Bevan et al., Nature 304:184-187 (1983)), the *bar* gene, which confers resistance to the herbicide phosphinothricin (White et al., Nucl. Acids Res 18: 1062 (1990), Spencer et al. Theor. Appl. Genet 79: 625-631 (1990)), the *hph* gene, which confers resistance to the antibiotic hygromycin (Blochinger & Diggelmann, Mol Cell Biol 4: 2929-2931), and the *dhfr* gene, which confers resistance to methatrexate (Bourouis et al., EMBO J. 2(7): 1099-1104 (1983)), and the EPSPS gene, which confers resistance to glyphosate (U.S. Patent Nos. 4,940,935 and 5,188,642).

1. Vectors Suitable for Agrobacterium Transformation

Many vectors are available for transformation using *Agrobacterium tumefaciens*. These typically carry at least one T-DNA border sequence and include vectors such as pBIN19 (Bevan, Nucl. Acids Res. (1984)) and pXYZ. Below, the construction of two typical vectors is described.

a. pCIB200 and pCIB2001:

The binary vectors pcIB200 and pCIB2001 are used for the construction of recombinant vectors for use with *Agrobacterium* and are constructed in the following manner. pTJS75kan is created by *NarI* digestion of pTJS75 (Schmidhauser & Helinski, J. Bacteriol. 164: 446-455 (1985)) allowing excision of the tetracycline-resistance gene, followed by insertion of an *AccI* fragment from pUC4K carrying an NPTII (Messing & Vierra,

Gene 19: 259-268 (1982): Bevan et al., Nature 304: 184-187 (1983): McBride et al., Plant Molecular Biology 14: 266-276 (1990)). *XhoI* linkers are ligated to the *EcoRV* fragment of PCIB7 which contains the left and right T-DNA borders, a plant selectable *nos/nptII* chimeric gene and the pUC polylinker (Rothstein et al., Gene 53: 153-161 (1987)), and the *XhoI*-digested fragment are cloned into *SalI*-digested pTJS75kan to create pCIB200 (see also EP 0 332 104, example 19). pCIB200 contains the following unique polylinker restriction sites: *EcoRI, SstI, KpnI, BglII, XbaI,* and *SalI.* pCIB2001 is a derivative of pCIB200 created by the insertion into the polylinker of additional restriction sites. Unique restriction sites in the polylinker of pCIB2001 are *EcoRI, SstI, KpnI, BglII, XbaI, SalI, MluI, BclI, AvrII, ApaI, HpaI,* and *StuI.* pCIB2001, in addition to containing these unique restriction sites also has plant and bacterial kanamycin selection, left and right T-DNA borders for *Agrobacterium*-mediated transformation, the RK2-derived *trfA* function for mobilization between *E. coli* and other hosts, and the *OriT* and *OriV* functions also from RK2. The pCIB2001 polylinker is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

b. pCIB10 and Hygromycin Selection Derivatives thereof:

The binary vector pCIB10 contains a gene encoding kanamycin resistance for selection in plants and T-DNA right and left border sequences and incorporates sequences from the wide host-range plasmid pRK252 allowing it to replicate in both *E. coli* and *Agrobacterium.* Its construction is described by Rothstein *et al.* (Gene 53: 153-161 (1987)). Various derivatives of pCIB10 are constructed which incorporate the gene for hygromycin B phosphotransferase described by Gritz *et al.* (Gene 25: 179-188 (1983)). These derivatives enable selection of transgenic plant cells on hygromycin only (pCIB743), or hygromycin and kanamycin (pCIB715, pCIB717).

2. Vectors Suitable for non-Agrobacterium Transformation

Transformation without the use of *Agrobacterium tumefaciens* circumvents the requirement for T-DNA sequences in the chosen transformation vector and consequently vectors lacking these sequences can be utilized in addition to vectors such as the ones described above which contain T-DNA sequences. Transformation techniques which do not rely on *Agrobacterium* include transformation via particle bombardment, protoplast uptake (*e.g.* PEG and electroporation) and microinjection. The choice of vector depends largely on the preferred selection for the species being transformed.

a. pCIB3064:

pCIB3064 is a pUC-derived vector suitable for direct gene transfer techniques in combination with selection by the herbicide basta (or phosphinothricin). The plasmid pCIB246 comprises the CaMV 35S promoter in operational fusion to the *E. coli* GUS gene and the CaMV 35S transcriptional terminator and is described in the PCT published application WO 93/07278. The 35S promoter of this vector contains two ATG sequences 5' of the start site. These sites are mutated using standard PCR techniques in such a way as to remove the ATGs and generate the restriction sites *Sspl* and *PvuII*. The new restriction sites are 96 and 37 bp away from the unique *SalI* site and 101 and 42 bp away from the actual start site. The resultant derivative of pCIB246 is designated pCIB3025. The GUS gene is then excised from pCIB3025 by digestion with *SalI* and *SacI*, the termini rendered blunt and religated to generate plasmid pCIB3060. The plasmid pJIT82 is obtained from the John Innes Centre, Norwich and the a 400 bp *SmaI* fragment containing the *bar* gene from *Streptomyces viridochromogenes* is excised and inserted into the *HpaI* site of pCIB3060 (Thompson *et al.* EMBO J 6: 2519-2523 (1987)). This generated pCIB3064, which comprises the *bar* gene under the control of the CaMV 35S promoter and terminator for herbicide selection, a gene for ampicillin resistance (for selection in *E. coli*) and a polylinker with the unique sites *SphI, PstI, HindIII,* and *BamHI*. This vector is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

b. pSOG19 and pSOG35:

pSOG35 is a transformation vector which utilizes the *E. coli* gene dihydrofolate reductase (DFR) as a selectable marker conferring resistance to methotrexate. PCR is used to amplify the 35S promoter (-800 bp), intron 6 from the maize Adh1 gene (-550 bp) and 18 bp of the GUS untranslated leader sequence from pSOG10. A 250-bp fragment encoding the *E. coli* dihydrofolate reductase type II gene is also amplified by PCR and these two PCR fragments are assembled with a *SacI-PstI* fragment from pB1221 (Clontech) which comprises the pUC19 vector backbone and the nopaline synthase terminator. Assembly of these fragments generates pSOG19 which contains the 35S promoter in fusion with the intron 6 sequence, the GUS leader, the DHFR gene and the nopaline synthase terminator. Replacement of the GUS leader in pSOG19 with the leader sequence from Maize Chlorotic Mottle Virus (MCMV) generates the vector pSOG35. pSOG19 and pSOG35 carry the pUC gene for ampicillin resistance and have *HindIII, SphI, PstI* and *EcoRI* sites available for the cloning of foreign substances.

B. Requirements for Construction of Plant Expression Cassettes

Gene sequences intended for expression in transgenic plants are first assembled in expression cassettes behind a suitable high expression level promoter and upstream of a suitable transcription terminator. These expression cassettes can then be easily transferred to the plant transformation vectors described above.

1. Promoter Selection

The selection of the promoter used in expression cassettes will determine the spatial and temporal expression pattern of the transgene in the transgenic plant. Selected promoters will express transgenes in specific cell types (such as leaf epidermal cells, mesophyll cells, root cortex cells) or in specific tissues or organs (roots, leaves or flowers, for example) and the selection will reflect the desired location of accumulation of the *NIM1* gene product or altered *NIM1* gene product. Alternatively, the selected promoter may drive expression of the gene under a light-induced or other temporally regulated promoter.

a. Constitutive Expression, the CaMV 35S Promoter:

Construction of the plasmid pCGN1761 is described in the published patent application EP 0 392 225 (example 23) which is hereby incorporated by reference. pCGN1761 contains the "double" 35S promoter and the *tml* transcriptional terminator with a unique *EcoRI* site between the promoter and the terminator and has a pUC-type backbone. A derivative of pCGN1761 is constructed which has a modified polylinker which includes *NotI* and *XhoI* sites in addition to the existing *EcoRI* site. This derivative is designated pCGN1761ENX. pCGN1761ENX is useful for the cloning of cDNA sequences or gene sequences (including microbial ORF sequences) within its polylinker for the purpose of their expression under the control of the 35S promoter in transgenic plants. The entire 35S promoter-gene sequence-*tml* terminator cassette of such a construction can be excised by *HindIII*, *SphI*, *SalI*, and *XbaI* sites 5' to the promoter and *XbaI*, *BamHI* and *BglI* sites 3' to the terminator for transfer to transformation vectors such as those described above. Furthermore, the double 35S promoter fragment can be removed by 5' excision with *HindIII*, *SphI*, *SalI*, *XbaI*, or *PstI*, and 3' excision with any of the polylinker restriction sites (*EcoRI*, *NotI* or *XhoI*) for replacement with another promoter.

b. Modification of pCGN1761ENX by Optimization of the Translational Initiation Site:

For any of the constructions described herein, modifications around the cloning sites can be made by the introduction of sequences which may enhance translation. This is particularly useful when overexpression is desired.

pCGN1761ENX is cleaved with *SphI*, treated with T4 DNA polymerase and religated, thus destroying the *SphI* site located 5' to the double 35S promoter. This generates vector pCGN1761ENX/Sph-. pCGN1761ENX/Sph- is cleaved with *EcoRI*, and ligated to an annealed molecular adaptor of the sequence 5'-AATTCTAAAGCATGCCGATCGG-3'/5'-AATTCCGATCGGCATGCTTTA-3' (SEQ ID NO's: 12 and 13). This generates the vector pCGNSENX, which incorporates the *quasi*-optimized plant translational initiation sequence TAAA-C adjacent to the ATG which is itself part of an *SphI* site which is suitable for cloning heterologous genes at their initiating methionine. Downstream of the *SphI* site, the *EcoRI*, *NotI*, and *XhoI* sites are retained.

An alternative vector is constructed which utilizes an *NcoI* site at the initiating ATG. This vector, designated pCGN1761NENX is made by inserting an annealed molecular adaptor of the sequence 5'-AATTCTAAACCATGGCGATCGG-3'/5'-AATTCCGATCGCCATGGTTTA-3' (SEQ ID NO's: 14 and 15) at the pCGN1761ENX *EcoRI* site. Thus the vector includes the *quasi*-optimized sequence TAAACC adjacent to the initiating ATG which is within the *NcoI* site. Downstream sites are *EcoRI*, *NotI*, and *XhoI*. Prior to this manipulation, however, the two *NcoI* sites in the pCGN1761ENX vector (at upstream positions of the 5' 35S promoter unit) are destroyed using similar techniques to those described above for *SphI* or alternatively using "inside-outside" PCR. Innes *et al.* PCR Protocols: A guide to methods and applications. Academic Press, New York (1990). This manipulation can be assayed for any possible detrimental effect on expression by insertion of any plant cDNA or reporter gene sequence into the cloning site followed by routine expression analysis in plants.

c. Expression under a Chemically/Pathogen Regulatable Promoter:

The double 35S promoter in pCGN1761ENX may be replaced with any other promoter of choice which will result in suitably high expression levels. By way of example, a chemically regulated PR-1 promoter, which is described in U.S. Patent No. 5,614,395, which is hereby incorporated by reference in its entirety, may replace the double 35S promoter. The promoter of choice is preferably excised from its source by restriction enzymes, but can alternatively be PCR-amplified using primers which carry appropriate terminal restriction sites. Should PCR-amplification be undertaken, then the promoter should be re-sequenced to check for amplification errors after the cloning of the amplified promoter in the target vector. The chemically/pathogen regulatable tobacco PR-1a promoter is cleaved from plasmid pCIB1004 (see EP 0 332 104, example 21 for construction which is hereby incorporated by reference) and transferred to plasmid pCGN1761ENX (Uknes et al. 1992). pCIB1004 is cleaved with *NcoI* and the resultant 3'

overhang of the linearized fragment is rendered blunt by treatment with T4 DNA polymerase. The fragment is then cleaved with *HindIII* and the resultant PR-1a-promoter-containing fragment is gel purified and cloned into pCGN1761ENX from which the double 35S promoter has been removed. This is done by cleavage with *XhoI* and blunting with T4 polymerase, followed by cleavage with *HindIII* and isolation of the larger vector-terminator containing fragment into which the pCIB1004 promoter fragment is cloned. This generates a pCGN1761ENX derivative with the PR-1a promoter and the *tml* terminator and an intervening polylinker with unique *EcoRI* and *NotI* sites. Selected *NIM1* genes can be inserted into this vector, and the fusion products (*i.e.* promoter-gene-terminator) can subsequently be transferred to any selected transformation vector, including those described in this application.

Various chemical regulators may be employed to induce expression of the *NIM1* coding sequence in the plants transformed according to the present invention. In the context of the instant disclosure, "chemical regulators" include chemicals known to be inducers for the PR-1 promoter in plants, or close derivatives thereof. A preferred group of regulators for the PR-1 promoter is based on the benzo-1,2,3-thiadiazole (BTH) structure and includes, but is not limited to, the following types of compounds: benzo-1,2,3-thiadiazolecarboxylic acid, benzo-1,2,3-thiadiazolethiocarboxylic acid, cyanobenzo-1,2,3-thiadiazole, benzo-1,2,3-thiadiazolecarboxylic acid amide, benzo-1,2,3-thiadiazolecarboxylic acid hydrazide, benzo-1,2,3-thiadiazole-7-carboxylic acid, benzo-1,2,3-thiadiazole-7-thiocarboxylic acid, 7-cyanobenzo-1,2,3-thiadiazole, benzo-1,2,3-thiadiazolecarboxylate in which the alkyl group contains one to six carbon atoms, methyl benzo-1,2,3-thiadiazole-7-carboxylate, n-propyl benzo-1,2,3-thiadiazole-7-carboxylate, benzyl benzo-1,2,3-thiadiazole-7-carboxylate, benzo-1,2,3-thiadiazole-7-carboxylic acid sec-butylhydrazide, and suitable derivatives thereof. Other chemical inducers may include, for example, benzoic acid, salicylic acid (SA), polyacrylic acid and substituted derivatives thereof; suitable substituents include lower alkyl, lower alkoxy, lower alkylthio, and halogen. Still another group of regulators for the chemically inducible DNA sequences of this invention is based on the pyridine carboxylic acid structure, such as the isonicotinic acid structure and preferably the haloisonicotinic acid structure. Preferred are dichloroisonicotinic acids and derivatives thereof, for example the lower alkyl esters. Suitable members of this class of regulator compounds are, for example, 2,6-dichloroisonicotinic acid (INA), and the lower alkyl esters thereof, especially the methyl ester.

d. Constitutive Expression, the Actin Promoter:

Several isoforms of actin are known to be expressed in most cell types and consequently the actin promoter is a good choice for a constitutive promoter. In particular, the promoter from the rice *Act1* gene has been cloned and characterized (McElroy *et al.* Plant Cell 2: 163-171 (1990)). A 1.3kb fragment of the promoter was found to contain all the regulatory elements required for expression in rice protoplasts. Furthermore, numerous expression vectors based on the *Act1* promoter have been constructed specifically for use in monocotyledons (McElroy *et al.* Mol. Gen. Genet. 231: 150-160 (1991)). These incorporate the *Act1*-intron 1, *Adh1* 5' flanking sequence and *Adh1*-intron 1 (from the maize alcohol dehydrogenase gene) and sequence from the CaMV 35S promoter. Vectors showing highest expression were fusions of 35S and *Act1* intron or the *Act1* 5' flanking sequence and the *Act1* intron. Optimization of sequences around the initiating ATG (of the GUS reporter gene) also enhanced expression. The promoter expression cassettes described by McElroy *et al.* (Mol. Gen. Genet. 231: 150-160 (1991)) can be easily modified for the expression of cellulase genes and are particularly suitable for use in monocotyledonous hosts. For example, promoter-containing fragments is removed from the McElroy constructions and used to replace the double 35S promoter in pCGN1761ENX, which is then available for the insertion of specific gene sequences. The fusion genes thus constructed can then be transferred to appropriate transformation vectors. In a separate report the rice *Act1* promoter with its first intron has also been found to direct high expression in cultured barley cells (Chibbar *et al.* Plant Cell Rep. 12: 506-509 (1993)).

e. Constitutive Expression, the Ubiquitin Promoter:

Ubiquitin is another gene product known to accumulate in many cell types and its promoter has been cloned from several species for use in transgenic plants (*e.g.* sunflower - Binet *et al.* Plant Science 79: 87-94 (1991) and maize - Christensen *et al.* Plant Molec. Biol. 12: 619-632 (1989)). The maize ubiquitin promoter has been developed in transgenic monocot systems and its sequence and vectors constructed for monocot transformation are disclosed in the patent publication EP 0 342 926 (to Lubrizol) which is herein incorporated by reference. Taylor *et al.* (Plant Cell Rep. 12: 491-495 (1993)) describe a vector (pAHC25) which comprises the maize ubiquitin promoter and first intron and its high activity in cell suspensions of numerous monocotyledons when introduced via microprojectile bombardment. The ubiquitin promoter is suitable for the expression of cellulase genes in transgenic plants, especially monocotyledons. Suitable vectors are derivatives of pAHC25 or any of the transformation vectors described in this application, modified by the introduction of the appropriate ubiquitin promoter and/or intron sequences.

f. Root Specific Expression:

Another pattern of expression for the *NIM1* gene of the instant invention is root expression. A suitable root promoter is described by de Framond (FEBS 290: 103-106 (1991)) and also in the published patent application EP 0 452 269 (to Ciba-Geigy) which is herein incorporated by reference. This promoter is transferred to a suitable vector such as pCGN1761ENX for the insertion of a cellulase gene and subsequent transfer of the entire promoter-gene-terminator cassette to a transformation vector of interest.

g. Wound-Inducible Promoters:

Wound-inducible promoters may also be suitable for expression of *NIM1* genes of the invention. Numerous such promoters have been described (*e.g.* Xu *et al.* Plant Molec. Biol. 22: 573-588 (1993), Logemann *et al.* Plant Cell 1: 151-158 (1989), Rohrmeier & Lehle, Plant Molec. Biol. 22: 783-792 (1993), Firek *et al.* Plant Molec. Biol. 22: 129-142 (1993), Warner *et al.* Plant J. 3: 191-201 (1993)) and all are suitable for use with the instant invention. Logemann *et al.* describe the 5' upstream sequences of the dicotyledonous potato *wun1* gene. Xu *et al.* show that a wound-inducible promoter from the dicotyledon potato (*pin2*) is active in the monocotyledon rice. Further, Rohrmeier & Lehle describe the cloning of the maize *Wip1* cDNA which is wound induced and which can be used to isolate the cognate promoter using standard techniques. Similar, Firek *et al.* and Warner *et al.* have described a wound-induced gene from the monocotyledon *Asparagus officinalis* which is expressed at local wound and pathogen invasion sites. Using cloning techniques well known in the art, these promoters can be transferred to suitable vectors, fused to the *NIM1* genes of this invention, and used to express these genes at the sites of plant wounding.

h. Pith-Preferred Expression:

Patent Application WO 93/07278 (to Ciba-Geigy) which is herein incorporated by reference describes the isolation of the maize *trpA* gene which is preferentially expressed in pith cells. The gene sequence and promoter extending up to -1726 bp from the start of transcription are presented. Using standard molecular biological techniques, this promoter, or parts thereof, can be transferred to a vector such as pCGN1761 where it can replace the 35S promoter and be used to drive the expression of a foreign gene in a pith-preferred manner. In fact, fragments containing the pith-preferred promoter or parts thereof can be transferred to any vector and modified for utility in transgenic plants.

i. Leaf-Specific Expression:

A maize gene encoding phosphoenol carboxylase (PEPC) has been described by Hudspeth & Grula (Plant Molec Biol 12: 579-589 (1989)). Using standard molecular biological techniques the promoter for this gene can be used to drive the expression of any gene in a leaf-specific manner in transgenic plants.

j. Expression with Chloroplast Targeting:

Chen & Jagendorf (J. Biol. Chem. 268: 2363-2367 (1993) have described the successful use of a chloroplast transit peptide for import of a heterologous transgene. This peptide used is the transit peptide from the *rbcS* gene from *Nicotiana plumbaginifolia* (Poulsen *et al.* Mol. Gen. Genet. 205: 193-200 (1986)). Using the restriction enzymes *DraI* and *SphI*. pr *Tsp509I* and *SphI* the DNA sequence encoding this transit peptide can be excised from the plasmid prbcS-8B and manipulated for use with any of the constructions described above. The *DraI-SphI* fragment extends from -58 relative to the initiating *rbcS* ATG to, and including, the first amino acid (also a methionine) of the mature peptide immediately after the import cleavage site, whereas the *Tsp509I-SphI* fragment extends from -8 relative to the initiating *rbcS* ATG to, and including, the first amino acid of the mature peptide.

Thus, these fragments can be appropriately inserted into the polylinker of any chosen expression cassette generating a transcriptional fusion to the untranslated leader of the chosen promoter (*e.g.* 35S, PR-1a, actin, ubiquitin *etc.*), while enabling the insertion of a *NIM1* gene in correct fusion downstream of the transit peptide. Constructions of this kind are routine in the art. For example, whereas the *DraI* end is already blunt, the 5' *Tsp509I* site may be rendered blunt by T4 polymerase treatment, or may alternatively be ligated to a linker or adaptor sequence to facilitate its fusion to the chosen promoter. The 3' *SphI* site may be maintained as such, or may alternatively be ligated to adaptor of linker sequences to facilitate its insertion into the chosen vector in such a way as to make available appropriate restriction sites for the subsequent insertion of a selected *NIM1* gene. Ideally the ATG of the *SphI* site is maintained and comprises the first ATG of the selected *NIM1* gene. Chen & Jagendorf provide consensus sequences for ideal cleavage for chloroplast import, and in each case a methionine is preferred at the first position of the mature protein. At subsequent positions there is more variation and the amino acid may not be so critical. In any case, fusion constructions can be assessed for efficiency of import *in vitro* using the methods described by Bartlett *et al.* (In: Edelmann *et al.* (Eds.) Methods in Chloroplast Molecular Biology, Elsevier pp 1081-1091 (1982)) and Wasmann *et al.* (Mol. Gen. Genet.

205: 446-453 (1986)). Typically the best approach may be to generate fusions using the selected *NIM1* gene or altered form of the *NIM1* gene with no modifications at the amino terminus, and only to incorporate modifications when it is apparent that such fusions are not chloroplast imported at high efficiency, in which case modifications may be made in accordance with the established literature (Chen & Jagendorf; Wasman *et al.*; Ko & Ko, J. Biol. Chem 267: 13910-13916 (1992)).

A preferred vector is constructed by transferring the *DraI-SphI* transit peptide encoding fragment from prbcS-8B to the cloning vector pCGN1761ENX/Sph-. This plasmid is cleaved with *EcoRI* and the termini rendered blunt by treatment with T4 DNA polymerase. Plasmid prbcS-8B is cleaved with *SphI* and ligated to an annealed molecular adaptor of the sequence 5'-CCAGCTGGAATTCCG-3'/5'-CGGAATTCCAGCTGGCATG-3' (SEQ ID NO's: 16 and 17). The resultant product is 5'-terminally phosphorylated by treatment with T4 kinase. Subsequent cleavage with *DraI* releases the transit peptide encoding fragment which is ligated into the blunt-end ex-*EcoRI* sites of the modified vector described above. Clones oriented with the 5' end of the insert adjacent to the 3' end of the 35S promoter are identified by sequencing. These clones carry a DNA fusion of the 35S leader sequence to the *rbcS-8A* promoter-transit peptide sequence extending from -58 relative to the *rbcS* ATG to the ATG of the mature protein, and including in that region a unique *SphI* site, and a newly created *EcoRI* site, as well as the existing *NotI* and *XhoI* sites of pCGN1761ENX. This new vector is designated pCGN1761/CT. DNA sequences are transferred to pCGN1761/CT in frame by amplification using PCR techniques and incorporation of an *SphI, NSphI,* or *NlaIII* site at the amplified ATG, which following restriction enzyme cleavage with the appropriate enzyme is ligated into *SphI*-cleaved pCGN1761/CT. To facilitate construction, it may be required to change the second amino acid of the product of the cloned gene; however, in almost all cases the use of PCR together with standard site directed mutagenesis will enable the construction of any desired sequence around the cleavage site and first methionine of the mature protein.

A further preferred vector is constructed by replacing the double 35S promoter of pCGN1761ENX with the *BamHI-SphI* fragment of prbcS-8A which contains the full-length, light-regulated *rbcS-8A* promoter from -1038 (relative to the transcriptional start site) up to the first methionine of the mature protein. The modified pCGN1761 with the destroyed *SphI* is cleaved with *PstI* and *EcoRI* and treated with T4 DNA polymerase to render termini blunt. prbcS-8A is cleaved with *SphI* and ligated to the annealed molecular adaptor of the sequence described above. The resultant product is 5'-terminally phosphorylated by treatment with T4 kinase. Subsequent cleavage with *BamHI* releases the promoter-transit peptide containing fragment which is treated with T4 DNA polymerase to render the *BamHI*

terminus blunt. The promoter-transit peptide fragment thus generated is cloned into the prepared pCGN1761ENX vector, generating a construction comprising the *rbcS-8A* promoter and transit peptide with an *SphI* site located at the cleavage site for insertion of heterologous genes. Further, downstream of the *SphI* site there are *EcoRI* (re-created), *NotI*, and *XhoI* cloning sites. This construction is designated pCGN1761rbcS/CT.

Similar manipulations can be undertaken to utilize other GS2 chloroplast transit peptide encoding sequences from other sources (monocotyledonous and dicotyledonous) and from other genes. In addition, similar procedures can be followed to achieve targeting to other subcellular compartments such as mitochondria.

2. Transcriptional Terminators

A variety of transcriptional terminators are available for use in expression cassettes. These are responsible for the termination of transcription beyond the transgene and its correct polyadenylation. Appropriate transcriptional terminators are those which are known to function in plants and include the CaMV 35S terminator, the *tml* terminator, the nopaline synthase terminator and the pea *rbcS* E9 terminator. These can be used in both monocotyledons and dicotyledons.

3. Sequences for the Enhancement or Regulation of Expression

Numerous sequences have been found to enhance gene expression from within the transcriptional unit and these sequences can be used in conjunction with the genes of this invention to increase their expression in transgenic plants.

Various intron sequences have been shown to enhance expression, particularly in monocotyledonous cells. For example, the introns of the maize *Adh1* gene have been found to significantly enhance the expression of the wild-type gene under its cognate promoter when introduced into maize cells. Intron 1 was found to be particularly effective and enhanced expression in fusion constructs with the chloramphenicol acetyltransferase gene (Callis *et al.*, Genes Develop. 1: 1183-1200 (1987)). In the same experimental system, the intron from the maize *bronze1* gene had a similar effect in enhancing expression. Intron sequences have been routinely incorporated into plant transformation vectors, typically within the non-translated leader.

A number of non-translated leader sequences derived from viruses are also known to enhance expression, and these are particularly effective in dicotyledonous cells. Specifically, leader sequences from Tobacco Mosaic Virus (TMV, the "W-sequence"), Maize Chlorotic Mottle Virus (MCMV), and Alfalfa Mosaic Virus (AMV) have been shown to be

effective in enhancing expression (*e.g.* Gallie *et al.* Nucl. Acids Res. 15: 8693-8711 (1987); Skuzeski *et al.* Plant Molec. Biol. 15: 65-79 (1990)).

4. Targeting of the Gene Product Within the Cell

Various mechanisms for targeting gene products are known to exist in plants and the sequences controlling the functioning of these mechanisms have been characterized in some detail. For example, the targeting of gene products to the chloroplast is controlled by a signal sequence found at the amino terminal end of various proteins which is cleaved during chloroplast import to yield the mature protein (*e.g.* Comai *et al.* J. Biol. Chem. 263: 15104-15109 (1988)). These signal sequences can be fused to heterologous gene products to effect the import of heterologous products into the chloroplast (van den Broeck, et al. Nature 313: 358-363 (1985)). DNA encoding for appropriate signal sequences can be isolated from the 5' end of the cDNAs encoding the RUBISCO protein, the CAB protein, the EPSP synthase enzyme, the GS2 protein and many other proteins which are known to be chloroplast localized.

Other gene products are localized to other organelles such as the mitochondrion and the peroxisome (*e.g.* Unger *et al.* Plant Molec. Biol. 13: 411-418 (1989)). The cDNAs encoding these products can also be manipulated to effect the targeting of heterologous gene products to these organelles. Examples of such sequences are the nuclear-encoded ATPases and specific aspartate amino transferase isoforms for mitochondria. Targeting cellular protein bodies has been described by Rogers *et al.* (Proc. Natl. Acad. Sci. USA 82: 6512-6516 (1985)).

In addition, sequences have been characterized which cause the targeting of gene products to other cell compartments. Amino terminal sequences are responsible for targeting to the ER, the apoplast, and extracellular secretion from aleurone cells (Koehler & Ho, Plant Cell 2: 769-783 (1990)). Additionally, amino terminal sequences in conjunction with carboxy terminal sequences are responsible for vacuolar targeting of gene products (Shinshi *et al.* Plant Molec. Biol. 14: 357-368 (1990)).

By the fusion of the appropriate targeting sequences described above to transgene sequences of interest it is possible to direct the transgene product to any organelle or cell compartment. For chloroplast targeting, for example, the chloroplast signal sequence from the RUBISCO gene, the CAB gene, the EPSP synthase gene, or the GS2 gene is fused in frame to the amino terminal ATG of the transgene. The signal sequence selected should include the known cleavage site, and the fusion constructed should take into account any amino acids after the cleavage site which are required for cleavage. In some cases this requirement may be fulfilled by the addition of a small number of amino acids between the

cleavage site and the transgene ATG or, alternatively, replacement of some amino acids within the transgene sequence. Fusions constructed for chloroplast import can be tested for efficacy of chloroplast uptake by *in vitro* translation of *in vitro* transcribed constructions followed by *in vitro* chloroplast uptake using techniques described by Bartlett *et al.* In: Edelmann *et al.* (Eds.) Methods in Chloroplast Molecular Biology, Elsevier pp 1081-1091 (1982) and Wasmann *et al.* Mol. Gen. Genet. 205: 446-453 (1986). These construction techniques are well known in the art and are equally applicable to mitochondria and peroxisomes.

The above-described mechanisms for cellular targeting can be utilized not only in conjunction with their cognate promoters, but also in conjunction with heterologous promoters so as to effect a specific cell-targeting goal under the transcriptional regulation of a promoter which has an expression pattern different to that of the promoter from which the targeting signal derives.

C. Transformation

Once the *NIM1* coding sequence has been cloned into an expression system, it is transformed into a plant cell. Plant tissues suitable for transformation include leaf tissues, root tissues, meristems, and protoplasts. The present system can be utilized in any plant which can be transformed and regenerated. Such methods for transformation and regeneration are well known in the art. Methodologies for the construction of plant expression cassettes as well as the introduction of foreign DNA into plants is generally described in the art. Generally, for the introduction of foreign DNA into plants, Ti plasmid vectors have been utilized for the delivery of foreign DNA. Also utilized for such delivery have been direct DNA uptake, liposomes, electroporation, micro-injection, and microprojectiles. Such methods had been published in the art. See, for example, Bilang et al. (1991) Gene 100: 247-250; Scheid et al., (1991) Mol. Gen. Genet. 228: 104-112; Guerche et al., (1987) Plant Science 52: 111-116; Neuhause et al., (1987) Theor. Appl. Genet. 75: 30-36; Klein et al., (1987) Nature 327: 70-73; Howell et al., (1980) Science 208:1265; Horsch et al., (1985) Science 227: 1229-1231; DeBlock et al., (1989) Plant Physiology 91: 694-701; Methods for Plant Molecular Biology (Weissbach and Weissbach, eds.) Academic Press, Inc. (1988); and Methods in Plant Molecular Biology (Schuler and Zielinski, eds.) Academic Press, Inc. (1989). See also US. patent No. 5,625,136 which are incorporated herein by reference in their entirety. It is understood that the method of transformation will depend upon the plant cell to be transformed. Transformation of tobacco, tomato, potato, and *Arabidopsis thaliana* using a binary Ti vector system. *Plant Physiol.* 81:301-305, 1986; Fry, J., Barnason, A., and Horsch, R.B. Transformation of

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Bacteria from the genus *Agrobacterium* can be utilized to transform plant cells. Suitable species of such bacterium include *Agrobacterium tumefaciens* and *Agrobacterium rhizogens. Agrobacterium tumefaciens* (e.g., strains LBA4404 or EHA105) is particularly useful due to its well-known ability to transform plants.

1. Transformation of Dicotyledons

Transformation techniques for dicotyledons are well known in the art and include *Agrobacterium*-based techniques and techniques which do not require *Agrobacterium*. Non-*Agrobacterium* techniques involve the uptake of exogenous genetic material directly by protoplasts or cells. This can be accomplished by PEG or electroporation mediated uptake, particle bombardment-mediated delivery, or microinjection. Examples of these techniques are described by Paszkowski *et al.*, EMBO J 3: 2717-2722 (1984), Potrykus *et al.*, Mol. Gen. Genet. 199: 169-177 (1985), Reich *et al.*, Biotechnology 4: 1001-1004 (1986), and Klein *et al.*, Nature 327: 70-73 (1987). In each case the transformed cells are regenerated to whole plants using standard techniques known in the art.

Agrobacterium-mediated transformation is a preferred technique for transformation of dicotyledons because of its high efficiency of transformation and its broad utility with many different species. The many crop species which are alfalfa and poplar (EP 0 317 511 (cotton), EP 0 249 432 (tomato, to Calgene), WO 87/07299 (*Brassica*, to Calgene),

US 4,795,855 (poplar)). *Agrobacterium* transformation typically involves the transfer of the binary vector carrying the foreign DNA of interest (*e.g.* pCIB200 or pCIB2001) to an appropriate *Agrobacterium* strain which may depend of the complement of *vir* genes carried by the host *Agrobacterium* strain either on a co-resident Ti plasmid or chromosomally (*e.g.* strain CIB542 for pCIB200 and pCIB2001 (Uknes *et al.* Plant Cell 5: 159-169 (1993)). The transfer of the recombinant binary vector to *Agrobacterium* is accomplished by a triparental mating procedure using *E. coli* carrying the recombinant binary vector, a helper *E. coli* strain which carries a plasmid such as pRK2013 and which is able to mobilize the recombinant binary vector to the target *Agrobacterium* strain. Alternatively, the recombinant binary vector can be transferred to *Agrobacterium* by DNA transformation (Höfgen & Willmitzer, Nucl. Acids Res. 16: 9877 (1988)).

Transformation of the target plant species by recombinant *Agrobacterium* usually involves co-cultivation of the *Agrobacterium* with explants from the plant and follows protocols well known in the art. Transformed tissue is regenerated on selectable medium carrying the antibiotic or herbicide resistance marker present between the binary plasmid T-DNA borders.

Another approach to transforming plant cells with a gene involves propelling inert or biologically active particles at plant tissues and cells. This technique is disclosed in U.S. Patent Nos. 4,945,050; 5,036,006; and 5,100,792 all to Sanford et al. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and afford incorporation within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the desired gene. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried yeast cells, dried bacterium or a bacteriophage, each containing DNA sought to be introduced) can also be propelled into plant cell tissue.

2. Transformation of Monocotyledons

Transformation of most monocotyledon species has now also become routine. Preferred techniques include direct gene transfer into protoplasts using PEG or electroporation techniques, and particle bombardment into callus tissue. Transformations can be undertaken with a single DNA species or multiple DNA species (*i.e.* co-transformation) and both these techniques are suitable for use with this invention. Co-transformation may have the advantage of avoiding complete vector construction and of generating transgenic plants with unlinked loci for the gene of interest and the selectable

marker, enabling the removal of the selectable marker in subsequent generations, should this be regarded desirable. However, a disadvantage of the use of co-transformation is the less than 100% frequency with which separate DNA species are integrated into the genome (Schocher *et al.* Biotechnology 4: 1093-1096 (1986)).

Patent Applications EP 0 292 435 ([1280/1281] to Ciba-Geigy), EP 0 392 225 (to Ciba-Geigy) and WO 93/07278 (to Ciba-Geigy) describe techniques for the preparation of callus and protoplasts from an elite inbred line of maize, transformation of protoplasts using PEG or electroporation, and the regeneration of maize plants from transformed protoplasts. Gordon-Kamm *et al.* (Plant Cell 2: 603-618 (1990)) and Fromm *et al.* (Biotechnology 8: 833-839 (1990)) have published techniques for transformation of A188-derived maize line using particle bombardment. Furthermore, application WO 93/07278 (to Ciba-Geigy) and Koziel *et al.* (Biotechnology 11: 194-200 (1993)) describe techniques for the transformation of elite inbred lines of maize by particle bombardment. This technique utilizes immature maize embryos of 1.5-2.5 mm length excised from a maize ear 14-15 days after pollination and a PDS-1000He Biolistics device for bombardment.

Transformation of rice can also be undertaken by direct gene transfer techniques utilizing protoplasts or particle bombardment. Protoplast-mediated transformation has been described for *Japonica*-types and *Indica*-types (Zhang *et al.* Plant Cell Rep 7: 379-384 (1988); Shimamoto *et al.* Nature 338: 274-277 (1989); Datta *et al.* Biotechnology 8: 736-740 (1990)). Both types are also routinely transformable using particle bombardment (Christou *et al.* Biotechnology 9: 957-962 (1991)).

Patent Application EP 0 332 581 (to Ciba-Geigy) describes techniques for the generation, transformation and regeneration of Pooideae protoplasts. These techniques allow the transformation of *Dactylis* and wheat. Furthermore, wheat transformation has been described by Vasil *et al.* (Biotechnology 10: 667-674 (1992)) using particle bombardment into cells of type C long-term regenerable callus, and also by Vasil *et al.* (Biotechnology 11: 1553-1558 (1993)) and Weeks *et al.* (Plant Physiol. 102: 1077-1084 (1993)) using particle bombardment of immature embryos and immature embryo-derived callus. A preferred technique for wheat transformation, however, involves the transformation of wheat by particle bombardment of immature embryos and includes either a high sucrose or a high maltose step prior to gene delivery. Prior to bombardment, any number of embryos (0.75-1 mm in length) are plated onto MS medium with 3% sucrose (Murashiga & Skoog, Physiologia Plantarum 15: 473-497 (1962)) and 3 mg/l 2,4-D for induction of somatic embryos, which is allowed to proceed in the dark. On the chosen day of bombardment, embryos are removed from the induction medium and placed onto the osmoticum (*i.e.* induction medium with sucrose or maltose added at the desired

concentration, typically 15%). The embryos are allowed to plasmolyze for 2-3 h and are then bombarded. Twenty embryos per target plate is typical, although not critical. An appropriate gene-carrying plasmid (such as pCIB3064 or pSG35) is precipitated onto micrometer size gold particles using standard procedures. Each plate of embryos is shot with the DuPont Biolistics® helium device using a burst pressure of ~1000 psi using a standard 80 mesh screen. After bombardment, the embryos are placed back into the dark to recover for about 24 h (still on osmoticum). After 24 hrs, the embryos are removed from the osmoticum and placed back onto induction medium where they stay for about a month before regeneration. Approximately one month later the embryo explants with developing embryogenic callus are transferred to regeneration medium (MS + 1 mg/liter NAA, 5 mg/liter GA), further containing the appropriate selection agent (10 mg/l basta in the case of pCIB3064 and 2 mg/l methotrexate in the case of pSOG35). After approximately one month, developed shoots are transferred to larger sterile containers known as "GA7s" which contain half-strength MS, 2% sucrose, and the same concentration of selection agent. Patent application 08/147,161 describes methods for wheat transformation and is hereby incorporated by reference.

More recently, tranformation of monocotyledons using *Agrobacterium* has been described. *See,* WO 94/00977 and U.S. Patent No. 5,591,616, both of which are incorporated herein by reference.

Breeding

The isolated gene fragment of the present invention or altered forms of the *NIM1* gene can be utilized to confer disease resistance to a wide variety of plant cells, including those of gymnosperms, monocots, and dicots. Although the gene can be inserted into any plant cell falling within these broad classes, it is particularly useful in crop plant cells, such as rice, wheat, barley, rye, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane.

The overexpression of the *NIM1* gene and mutants thereof necessary for constitutive expression of SAR genes, in combination with other characteristics important for production and quality, can be incorporated into plant lines through breeding. Thus a further embodiment of the present invention is a method of producing transgenic

descendants of a transgenic parent plant comprising an isolated DNA molecule encoding an altered form of a NIM1 protein according to the invention comprising transforming said parent plant with a recombinant vector molecule according to the invention and transferring the trait to the descendants of said transgenic parent plant involving known plant breeding techniques.

Breeding approaches and techniques are known in the art. See, for example, Welsh J. R., Fundamentals of Plant Genetics and Breeding, John Wiley & Sons, NY (1981); Crop Breeding, Wood D. R. (Ed.) American Society of Agronomy Madison, Wisconsin (1983); Mayo O., The Theory of Plant Breeding, Second Edition, Clarendon Press, Oxford (1987); Singh, D.P., Breeding for Resistance to Diseases and Insect Pests, Springer-Verlag, NY (1986); and Wricke and Weber, Quantitative Genetics and Selection Plant Breeding, Walter de Gruyter and Co., Berlin (1986).

Propagation of genetic properties engineered into the transgenic seeds and plants and maintainance in descendant plants

The genetic properties engineered into the transgenic seeds and plants described above are passed on by sexual reproduction or vegetative growth and can thus be maintained and propagated in descendant plants. Generally said maintenance and propagation make use of known agricultural methods developed to fit specific purposes such as tilling, sowing or harvesting. Specialized processes such as hydroponics or greenhouse technologies can also be applied. As the growing crop is vulnerable to attack and damages caused by insects or infections as well as to competition by weed plants, measures are undertaken to control weeds, plant diseases, insects, nematodes, and other adverse conditions to improve yield. These include mechanical measures such a tillage of the soil or removal of weeds and infected plants, as well as the application of agrochemicals such as herbicides, fungicides, gametocides, nematicides, growth regulants, ripening agents and insecticides.

Use of the advantageous genetic properties of the transgenic plants and seeds according to the invention can further be made in plant breeding which aims at the development of plants with improved properties such as tolerance of pests, herbicides, or stress, improved nutritional value, increased yield, or improved structure causing less loss from lodging or shattering. The various breeding steps are characterized by well-defined human intervention such as selecting the lines to be crossed, directing pollination of the parental lines, or selecting appropriate descendant plants. Depending on the desired properties different breeding measures are taken. The relevant techniques are well known in the art

and include but are not limited to hybridization, inbreeding, backcross breeding, multiline breeding, variety blend, interspecific hybridization, aneuploid techniques, etc. Hybridization techniques also include the sterilization of plants to yield male or female sterile plants by mechanical, chemical or biochemical means. Cross pollination of a male sterile plant with pollen of a different line assures that the genome of the male sterile but female fertile plant will uniformly obtain properties of both parental lines. Thus, the transgenic seeds and plants according to the invention can be used for the breeding of improved plant lines which for example increase the effectiveness of conventional methods such as herbicide or pestidice treatment or allow to dispense with said methods due to their modified genetic properties. Alternatively new crops with improved stress tolerance can be obtained which, due to their optimized genetic "equipment", yield harvested product of better quality than products which were not able to tolerate comparable adverse developmental conditions.

In seeds production germination quality and uniformity of seeds are essential product characteristics, whereas germination quality and uniformity of seeds harvested and sold by the farmer is not important. As it is difficult to keep a crop free from other crop and weed seeds, to control seedborne diseases, and to produce seed with good germination, fairly extensive and well-defined seed production practices have been developed by seed producers, who are experienced in the art of growing, conditioning and marketing of pure seed. Thus, it is common practice for the farmer to buy certified seed meeting specific quality standards instead of using seed harvested from his own crop. Propagation material to be used as seeds is customarily treated with a protectant coating comprising herbicides, insecticides, fungicides, bactericides, nematicides, molluscicides or mixtures thereof. Customarily used protectant coatings comprise compounds such as captan, carboxin, thiram (TMTD®), methalaxyl (Apron®), and pirimiphos-methyl (Actellic®). If desired these compounds are formulated together with further carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation to provide protection against damage caused by bacterial, fungal or animal pests. The protectant coatings may be applied by impregnating propagation material with a liquid formulation or by coating with a combined wet or dry formulation. Other methods of application are also possible such as treatment directed at the buds or the fruit.

It is a further aspect of the present invention to provide new agricultural methods such as the methods examplified above which are characterized by the use of transgenic plants, transgenic plant material, or transgenic seed according to the present invention.

The seeds may be provided in a bag, container or vessel comprised of a suitable packaging material, the bag or container capable of being closed to contain seeds. The bag, container or vessel may be designed for either short term or long term storage, or both, of the seed. Examples of a suitable packaging material include paper, such as kraft paper, rigid or pliable plastic or other polymeric material, glass or metal. Desirably the bag, container, or vessel is comprised of a plurality of layers of packaging materials, of the same or differing type. In one embodiment the bag, container or vessel is provided so as to exclude or limit water and moisture from contacting the seed. In one example, the bag, container or vessel is sealed, for example heat sealed, to prevent water or moisture from entering. In another embodiment water absorbent materials are placed between or adjacent to packaging material layers. In yet another embodiment the bag, container or vessel, or packaging material of which it is comprised is treated to limit, suppress or prevent disease, contamination or other adverse affects of storage or transport of the seed. An example of such treatment is sterilization, for example by chemical means or by exposure to radiation. Comprised by the present invention is a commercial bag comprising seed of a transgenic plant comprising at least one altered form of a NIM1 protein or a NIM1 protein that is expressed in said transformed plant at higher levels than in a wild type plant, together with a suitable carrier, together with lable instructions for the use thereof for conferring broad spectrum disease resistance to plants.

Disease Resistance

Disease Resistance evaluation is performed by methods known in the art. For examples see, Uknes et al, (1993) Molecular Plant Microbe Interactions 6: 680-685; Gorlach et al., (1996) Plant Cell 8:629-643; Alexander et al., Proc. Natl. Acad. Sci. USA 90: 7327-7331.

A. *Phytophthora parasitica* (Black shank) Resistance Assay

Assays for resistance to *Phytophthora parasitica*, the causative organism of black shank, are performed on six-week-old plants grown as described in Alexander et al., Proc. Natl. Acad. Sci. USA 90: 7327-7331. Plants are watered, allowed to drain well, and then inoculated by applying 10 ml of a sporangium suspension (300 sporangia/ml) to the soil. Inoculated plants are kept in a greenhouse maintained at 23-25°C day temperature, and 20-22°C night temperature. The wilt index used for the assay is as follows: 0=no symptoms; 1=no symptoms; 1=some sign of wilting, with reduced turgidity; 2=clear wilting symptoms, but no rotting or stunting; 3=clear wilting symptoms with stunting, but no apparent stem rot;

4=severe wilting, with visible stem rot and some damage to root system; 5=as for 4, but plants near death or dead, and with severe reduction of root system. All assays are scored blind on plants arrayed in a random design.

B. *Pseudomonas syringae* Resistance Assay

Pseudomonas syringae pv. tabaci strain #551 is injected into the two lower leaves of several 6-7-week-old plants at a concentration of 10^6 or 3 x 10^6 per ml in H_20. Six individual plants are evaluated at each time point. *Pseudomonas tabaci* infected plants are rated on a 5 point disease severity scale, 5=100% dead tissue, 0=no symptoms. A T-test (LSD) is conducted on the evaluations for each day and the groupings are indicated after the Mean disease rating value. Values followed by the same letter on that day of evaluation are not statistically significantly different.

C. *Cercospora nicotianae* Resistance Assay

A spore suspension of *Cercospora nicotianae* (ATCC #18366) (100,000-150,000 spores per ml) is sprayed to imminent run-off onto the surface of the leaves. The plants are maintained in 100% humidity for five days. Thereafter the plants are misted with water 5-10 times per day. Six individual plants are evaluated at each time point. *Cercospora nicotianae* is rated on a % leaf area showing disease symptoms basis. A T-test (LSD) is conducted on the evaluations for each day and the groupings are indicated after the Mean disease rating value. Values followed by the same letter on that day of evaluation are not statistically significantly different.

D. *Peronospora parasitica* Resistance Assay

Assays for resistance to *Peronospora parasitica* are performed on plants as described in Uknes et al, (1993). Plants are inoculated with a combatible isolate of *P. parasitica* by spraying with a conidial suspension (approximately 5 x 10^4 spores per milliliter). Inoculated plants are incubated under humid conditions at 17° C in a growth chamber with a 14-hr day/10-hr night cycle. Plants are examined at 3-14 days, preferably 7-12 days, after inoculation for the presence of conidiophores. In addition, several plants from each treatment are randomly selected and stained with lactophenol-trypan blue (Keogh *et al.*, *Trans. Br. Mycol. Soc.* 74: 329-333 (1980)) for microscopic examination.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the effect of chemical inducers on the induction of SAR gene expression in wild-type and *nim1* plants. Chemical induction of SAR genes is diminished in *nim1* plants. Water, SA, INA, or BTH is applied to wild type (WT) and *nim1* plants. After 3 days, RNA is prepared from these plants and examined for expression of PR-1, PR-2, and PR-5.

FIGURE 2 depicts PR-1 gene expression in pathogen-infected Ws-O and *nim1* plants. Pathogen induction of PR-1 is diminished in *nim1* plants. Wild type (WT) and *nim1* plants were spray-inoculated with the Emwa race of *P. parasitica*. Samples were collected at days 0, 1, 2, 4, and 6 and RNA is analyzed by blot hybridization with an *A. thaliana* PR-1 cDNA probe to measure PR-1 mRNA accumulation.

FIGURE 3 shows the accumulation of PR-1 mRNA in *nim1* mutants and wild-type plants after pathogen infection or chemical treatment. Plants containing *nim1* alleles *nim1-1, -2, -3, -4, -5,* and *-6* and Ws-O (Ws) were treated with water (C), SA, INA, or BTH 3 days before RNA isolation. The Emwa sample consists of RNA isolated from plants 14 days post-inoculation with the Emwa isolate of *P. parasitica*. Blots were hybridized using an *Arabidopsis* PR-1 cDNA as a probe (Uknes *et al.*, 1992).

FIGURE 4 shows the levels of SA accumulation in Ws-O and *nim1* plants infected with *P. syringae*. *nim1* plants accumulate SNA following pathogen exposure. Leaves of wild type and *nim1* plantsare infiltrated with *Pst* DC3000(*avrRpt2*) or carrier medium (10 mM $MgCl_2$) alone. After 2 days, samples were collected from untreated, $MgCl_2$-treated, and DC3000(*avrRpt2*)-treated plants. Bacteria-treated samples were separated into primary (infiltrated) and secondary (noninfiltrated) leaves. Free SA and total SA following hydrolysis with β-glucosidase were quantified by HPLC. Error bars indicate SD of three replicate samples.

FIGURES 5A-D present a global map at increasing levels of resolution of the chromosomal region centered on *NIM1* with recombinants indicated, including, BACs, YACs and Cosmids in *NIM1* region.

(A) Map position of *NIM1* on chromosome 1. The total number of gametes scored is 2276.

(B) Yeast artificial chromosome (striped), bacterial artificial chromosome (BAC), and P1 clones used to clone *NIM1*.

(C) Cosmid clones that cover the *NIM1* locus. The three cosmids that complement *nim1-1* are shown as thicker lines.

(D) The four putative gene regions on the smallest fragment of complementing genomic DNA. The four open reading frames that comprise the *NIM1* gene are indicated by the open bars. The arrows indicate the direction of transcription. Numbering is relative to the first base of *Arabidopsis* genomic DNA present in cosmid D7.

FIGURE 6 shows the nucleic acid sequence of the *NIM1* gene and the amino acid sequence of the *NIM1* gene product, including changes in the various alleles. This nucleic acid sequence, which is on the opposite strand as the 9.9 kb sequence presented in SEQ ID NO:1, is also presented in SEQ ID NO:2, and the amino acid sequence of the *NIM1* gene product is also presented in SEQ ID NO:3.

FIGURE 7 shows the accumulation of *NIM1* induced by INA, BTH, SA and pathogen treatment in wild type plants and mutant alleles of *nim1*. The RNA gel blots in Figure 3 were probed for expression of RNA by using a probe derived from 2081 to 3266 in the sequence shown in Figure 6.

FIGURE 8 is an amino acid sequence comparison of Expressed Sequence Tag regions of the NIM1 protein and cDNA protein products of 4 rice gene sequences (SEQ ID NOs: 4-11); numbers correspond to amino acid positions in SEQ ID NO:3).

FIGURE 9 is a sequence alignment of the NIM1 protein sequence with IκBα from mouse, rat, and pig. Vertical bars (|) above the sequences indicate amino acid identity between NIM1 and the IκBα sequences (matrix score equals 1.5); double dots (:) above the sequences indicate a similarity score >0.5; single dots (.) above the sequences indicate a similarity score <0.5 but >0.0; and a score <0.0 indicates no similarity and has no indicia above the sequences (*see* Examples). Locations of the mammalian IκBα ankyrin domains were identified according to de Martin et al., *Gene* 152, 253-255 (1995). The dots within a sequence indicate gaps between NIM1 and IκBα proteins. The five ankyrin repeats in IκBα are indicated by the dashed lines under the sequence. Amino acids are numbered relative to the NIM1 protein with gaps introduced where appropriate. Plus signs (+) are placed above the sequences every 10 amino acids.

DEPOSITS

The following vector molecules have been deposited with American Type Culture Collection 12301 Parklawn Drive Rockville, MD 20852, U.S.A. on the dates indicated below:
Plasmid BAC-04 was deposited with ATCC on May 8, 1996 as ATCC 97543.
Plasmid P1-18 was deposited with ATCC on June 13, 1996 as ATCC 97606.
Cosmid D7 was deposited with ATCC on September 25, 1996 as ATCC 97736.

BRIEF DESCRIPTION OF THE SEQUENCES IN THE SEQUENCE LISTING

SEQ ID NO: 1 - 9919-bp genomic sequence of *NIM1* gene region 2 in Figure 5D .
SEQ ID NO: 2 - 5655-bp genomic sequence in Figure 6 (opposite strand from SEQ ID NO:1). comprising the coding region of the wild-type *Arabidopsis thaliana NIM1* gene.
SEQ ID NO: 3 - AA sequence of wild-type NIM1 protein encoded by cds of SEQ ID NO:2.
SEQ ID NO: 4 - Rice-1 AA sequence 33-155 from Figure 8.
SEQ ID NO: 5 - Rice-1 AA sequence 215-328 from Figure 8.
SEQ ID NO: 6 - Rice-2 AA sequence 33-155 from Figure 8.
SEQ ID NO: 7 - Rice-2 AA sequence 208-288 from Figure 8.
SEQ ID NO: 8 - Rice-3 AA sequence 33-155 from Figure 8.
SEQ ID NO: 9 - Rice-3 AA sequence 208-288 from Figure 8.
SEQ ID NO: 10 - Rice-4 AA sequence 33-155 from Figure 8.
SEQ ID NO: 11 - Rice-4 AA sequence 215-271 from Figure 8.
SEQ ID NO: 12 - Oligonucleotide.
SEQ ID NO: 13 - Oligonucleotide.
SEQ ID NO: 14 - Oligonucleotide.
SEQ ID NO: 15 - Oligonucleotide.
SEQ ID NO: 16 - Oligonucleotide.
SEQ ID NO: 17 - Oligonucleotide.
SEQ ID NO: 18 is the mouse IκBα amino acid sequence from Figure 8.
SEQ ID NO: 19 is the rat IκBα amino acid sequence from Figure 8.
SEQ ID NO: 20 is the pig IκBα amino acid sequence from Figure 8.
SEQ ID NO: 21 is the cDNA sequence of the *Arabidopsis thaliana NIM1* gene.
SEQ ID NO's: 22 and 23 are the DNA coding sequence and encoded amino acid sequence, respectively, of a dominant-negative form of the NIM1 protein having alanine residues instead of serine residues at amino acid positions 55 and 59.

SEQ ID NO's: 24 and 25 are the DNA coding sequence and encoded amino acid sequence, respectively, of a dominant-negative form of the NIM1 protein having an N-terminal deletion.

SEQ ID NO's: 26 and 27 are the DNA coding sequence and encoded amino acid sequence, respectively, of a dominant-negative form of the NIM1 protein having a C-terminal deletion.

SEQ ID NO's: 28 and 29 are the DNA coding sequence and encoded amino acid sequence, respectively, of an altered form of the *NIM1* gene having both N-terminal and C-terminal amino acid deletions.

SEQ ID NO's: 30 and 31 are the DNA coding sequence and encoded amino acid sequence, respectively, of the ankyrin domain of *NIM1*.

SEQ ID NOs:32 through 39 are oligonucleotide primers.

Definitions

acd:	accelerated cell death mutant plant
AFLP:	Amplified Fragment Length Polymorphism
avrRpt2:	avirulence gene Rpt2, isolated from *Pseudomonas syringae*
BAC:	Bacterial Artificial Chromosome
BTH:	benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester
CIM:	Constitutive IMmunity phenotype (SAR is constitutively activated)
cim:	constitutive immunity mutant plant
cM:	centimorgans
cpr1:	constitutive expresser of PR genes mutant plant
Col-O:	*Arabidopsis* ecotype Columbia
ECs:	Enzyme combinations
Emwa:	*Peronospora parasitica* isolate compatible in the Ws-O ecotype of Arabidopsis
EMS:	ethyl methane sulfonate
INA:	2,6-dichloroisonicotinic acid
Ler:	*Arabidopsis* ecotype *Landsberg erecta*
lsd:	lesions simulating disease mutant plant
nahG:	salicylate hydroxylase *Pseudomonas putida* that converts salicylic acid to catechol
NahG:	*Arabidopsis* line transformed with *nahG* gene
ndr:	non-race-specific disease resistance mutant plant

nim: non-inducible immunity mutant plant

NIM1: the wild type gene, involved in the SAR signal transduction cascade

NIM1: Protein encoded by the wild type *NIM1* gene

nim1: mutant allele of *NIM1*, conferring disease susceptibility to the plant; also refers to mutant *Arabidopsis thaliana* plants having the *nim1* mutant allele of *NIM1*

Noco: *Peronospora parasitica* isolate compatible in the Col-O ecotype of Arabidopsis

ORF: open reading frame

PCs: Primer combinations

PR: Pathogenesis Related

SA: salicylic acid

SAR: Systemic Acquired Resistance

SSLP: Simple Sequence Length Polymorphism

UDS: Universal Disease Susceptible phenotype

Wela: *Peronospora parasitica* isolate compatible in the Weiningen ecotype of Arabidopsis

Ws-O: *Arabidopsis* ecotype Issilewskija

WT: wild type

YAC: Yeast Artificial Chromosome

EXAMPLES

The invention is illustrated in further detail by the following detailed procedures, preparations, and examples. The examples are for illustration only, and are not to be construed as limiting the scope of the present invention.

Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by Sambrook, *et al.*, Molecular Cloning, eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989) and by T.J. Silhavy, M.L. Berman, and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and by Ausubel, F.M. *et al.*, Current Protocols in Molecular Biology, pub. by Greene Publishing Assoc. and Wiley-Interscience (1987).

A. Characterization of *nim1* Mutants

Example 1: Plant Lines and Fungal Strains

Arabidopsis thaliana ecotype Isilewskija (Ws-O; stock number CS 2360) and fourth-generation (T_4) seeds from T-DNA-transformed lines were obtained from the Ohio State University Arabidopsis Biological Resource Center (Columbus, OH). Second generation (M_2) seeds from ethyl methane sulfonate (EMS) mutagenized Ws-O plants were obtained from Lehle Seeds (Round Rock, TX).

Pseudomonas syringae pv. *Tomato* (*Pst*) strain DC3000 containing the cloned *avrRpt2* gene [DC3000(*avrRpt2*)] was obtained from B. Staskawicz, University of California, Berkeley. *P. parasitica* pathovars and their sources were as follows: Emwa from E. Holub and I.R. Crute, Horticultural Research Station, East Malling, Kent; Wela from A. Slusarenko and B. Mauch-Mani, Institut für Pflanzenbiologie, Zürich, Switzerland; and Noco from J. Parker, Sainsbury Laboratory, Norwich, England. Fungal cultures were maintained by weekly culturing on *Arabidopsis* ecotype Ws-O, Weiningen, and Col-O, for *P. parasitica* pathovars Emwa, Wela, and Noco, respectively.

Example 2: Mutant Screens

M_2 or T_4 seeds were grown on soil for 2 weeks under 14 hr of light per day, misted with 0.33 mM INA (0.25 mg/ml made from 25% INA in wettable powder; Ciba, Basel, Switzerland), and inoculated 4 days later by spraying a *P. parasitica* conidial suspension containing 5-10 $\times$ 10^4 conidiospores per ml of water. This fungus is normally virulent on the Arabidopsis Ws-O ecotype, unless resistance is first induced in these plants with isonicotinic acid (INA) or a similar compound. Plants were kept under humid conditions at 18°C for 1 week and then scored for fungal sporulation. Plants that supported fungal growth after INA treatment were selected as putative mutants.

Following incubation in a high humidity environment, plants with visible disease symptoms were identified, typically 7 days after the infection. These plants did not show resistance to the fungus, despite the application of the resistance-inducing chemical and were thus potential *nim* (noninducible-immunity) mutant plants. From 360,000 plants, 75 potential *nim* mutants were identified.

These potential mutant plants were isolated from the flat, placed under low humidity conditions and allowed to set seed. Plants derived from this seed were screened in an identical manner for susceptibility to the fungus Emwa, again after pretreatment with INA. The descendant plants that showed infection symptoms were defined as *nim* mutants. Six *nim* lines were thus identified. One line (*nim1-1*) was isolated from the T-DNA population and five (*nim1-2, nim1-3, nim1-4, nim1-5,* and *nim1-6*) from the EMS population.

Example 3: Disease Resistance of *nim1* Plants

Salicylic acid (SA) and benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) are chemicals that, like INA, induce broad spectrum disease resistance (SAR) in wild type plants. Mutant plants were treated with SA, INA, and BTH and then assayed for resistance to *Peronospora parasitica*. *P. parasitica* isolate 'Emwa' is a *P.p.* isolate that is compatible in the Ws ecotype. Compatible isolates are those that are capable of causing disease on a particular host. The *P. parasitica* isolate 'Noco' is incompatible on Ws but compatible on the Columbia ecotype. Incompatible pathogens are recognized by the potential host, eliciting a host response that prevents disease development.

Wild-type seeds and seeds for each of the *nim1* alleles (*nim1-1, -2, -3, -4, -5, -6*) were sown onto MetroMix 300 growing media, covered with a transparent plastic dome, and placed at 4°C in the dark for 3 days. After 3 days of 4°C treatment, the plants were moved to a phytotron for 2 weeks. By approximately 2 weeks post-planting, germinated seedlings had produced 4 true leaves. Plants were then treated with H_2O, 5mM SA, 300 µM BTH ,or 300 µM INA. Chemicals were applied as a fine mist to completely cover the seedlings using a chromister. Water control plants were returned to the growing phytotron while the chemically treated plants were held in a separate but identical phytotron. At 3 days post-chemical application, water and chemically treated plants were inoculated with the compatible 'Emwa' isolate. 'Noco' inoculation was conducted on water treated plants only. Following inoculation, plants were covered with a clear plastic dome to maintain high humidity required for successful *P. parasitica* infection and placed in a growing chamber with 19°C day/17° C night temperatures and 8h light/16h dark cycles.

To determine the relative strength of the different *nim1* alleles, each mutant was microscopically analyzed at various timepoints after inoculation for the growth of *P. parasitica* under normal growth conditions and following pretreatment with either SA, INA, or BTH. Under magnification, sporulation of the fungus could be observed at very early stages of disease development. The percentage of plants/pot showing sporulation at 5d, 6d, 7d, 11d and 14d after inoculation was determined and the density of sporulation was also recorded.

Table 1 shows, for each of the *nim1* mutant plant lines, the percent of plants that showed some surface conidia on at least one leaf after infection with the Emwa race of *P. parasitica*. *P. parasitica* was inoculated onto the plants three days after water or chemical treatment. The table indicates the number of days after infection that the disease resistance was rated.

Table 1

Percent Infection - Emwa/Control

mutant	Day 0	Day 5	Day 6	Day 7	Day 11
Ws WT	0	10	25	100	90
nim1-1	0	75	95	100	100
nim1-2	0	30	85	100	100
nim1-3	0	30	90	100	100
nim1-4	0	80	100	100	100
nim1-5	0	0	5	100	100
nim1-6	0	5	70	80	100

Percent Infection - Emwa/SA

mutant	Day 0	Day 5	Day 6	Day 7	Day 11
Ws WT	0	5	30	70	100
nim1-1	0	5	95	100	100
nim1-2	0	5	95	100	100
nim1-3	0	10	90	100	100
nim1-4	0	75	100	100	100
nim1-5	0	0	20	75	100
nim1-6	0	80	100	100	100

Percent Infection - Emwa/INA

mutant	Day 0	Day 5	Day 6	Day 7	Day 11
Ws WT	0	0	0	0	0
nim1-1	0	5	80	100	100
nim1-2	0	15	95	100	100
nim1-3	0	10	60	100	100
nim1-4	0	80	100	100	100
nim1-5	0	0	0	5	5
nim1-6	0	1	50	90	100

Percent Infection - Emwa/BTH

mutant	Day 0	Day 5	Day 6	Day 7	Day 11
Ws WT	0	0	0	0	0
nim1-1	0	1	5	30	100
nim1-2	0	0	25	90	100
nim1-3	0	15	70	100	100
nim1-4	0	80	100	100	100
nim1-5	0	0	1	1	10
nim1-6	0	1	90	100	100

As shown in Table 1, during normal growth, *nim1-1*, *nim1-2*, *nim1-3*, *nim1-4*, and *nim1-6* all supported approximately the same rate of fungal growth, which was somewhat faster than the Ws-0 control. The exception was the *nim1-5* plants where fungal growth was delayed by several days relative to both the other *nim1* mutants and the Ws-0 control, but eventually all of the *nim1-5* plants succumbed to the fungus.

Following SA treatment, the mutants could be grouped into three classes: *nim1-4* and *nim1-6* showed a relatively rapid fungal growth; *nim1-1*, *nim1-2*, *nim1-3* plants exhibited a somewhat slower rate of fungal growth; and fungal growth in *nim1-5* plants was even slower than in the untreated Ws-0 controls. Following either INA or BTH treatment, the mutants also fell into three classes where *nim1-4* was the most severely compromised in its ability to restrict fungal growth following chemical treatment; *nim1-1*, *nim1-2*, *nim1-3*, and *nim1-6* were all moderately compromised; and *nim1-5* was only slightly compromised. In these experiments, Ws-0 did not support fungal growth following INA or BTH treatment. Thus, with respect to inhibition of fungal growth following chemical treatment, the mutants fell into three classes with *nim1-4* being the most severely compromised, *nim1-1*, *nim1-2*, *nim1-3* and *nim1-6* showing an intermediate inhibition of fungus and *nim1-5* with only slightly impaired fungal resistance.

Table 2 shows the disease resistance assessment via infection rating of the various *nim1* alleles as well as of NahG plants at 7 and 11 days after innoculation with *Peronospora parasitica*. WsWT indicates the Ws wild type parent line in which the *nim1* alleles were found. The various *nim1* alleles are indicated in the table and the NahG plant is indicated also.

A description of the NahG plant has been previously published. (Delaney et al., Science 266, pp. 1247-1250 (1994)). NahG *Arabidopsis* is also described in U.S. Patent Application Serial No. 08/454,876, incorporated by reference herein. *nahG* is a gene from *Pseudomonas putida* encoding a salicylate hydroxylase that converts salicylic acid to catechol, thereby eliminating the accumulation of salicylic acid, a necessary signal transduction component for SAR in plants. Thus, NahG *Arabidopsis* plants do not display normal SAR, and they show much greater susceptibility in general to pathogens. However, the NahG plants still respond to the chemical inducers INA and BTH. NahG plants therefore serve as a kind of universal susceptibility control.

Table 2

Infection Severity - Emwa/Water

mutant	Day 7	Day 11
Ws WT	3	3
nim1-1	4	4.5
nim1-2	3	4
nim1-3	4	4
nim1-4	5	5
nim1-5	1	3.5
nim1-6	3	4.5
NahG	4	5

Infection Severity - Emwa/SA

mutant	Day 7	Day 11
Ws WT	3	4
nim1-1	3	4.5
nim1-2	3	4
nim1-3	3	4
nim1-4	4	5
nim1-5	3	3
nim1-6	4	4.5
NahG	4	5

Infection Severity - Emwa/INA

mutant	Day 7	Day 11
Ws WT	0	0
nim1-1	2.5	4
nim1-2	4	4
nim1-3	3	3.5
nim1-4	4	5
nim1-5	1	2
nim1-6	3	4.5
NahG	3	3

Infection Severity - Emwa/BTH

mutant	Day 7	Day 11
Ws WT	0	0
nim1-1	2.5	4
nim1-2	3.5	4
nim1-3	3	3.5
nim1-4	4	5
nim1-5	1.5	2
nim1-6	3	4
NahG	0	0

From Table 2 it can be seen that the *nim1-4* and *nim1-6* alleles had the most severe *Peronospora parasitica* infections; this was most easily observable at the earlier time points. In addition, the *nim1-5* allele showed the greatest response to both INA and BTH and therefore was deemed the weakest *nim1* allele. The NahG plants showed very good response to both INA and BTH and looked very similar to the *nim1-5* allele. However, at late time points, Day 11 in the Table, the disease resistance induced in the NahG plants began to fade, and there was a profound difference between INA and BTH in that the INA-induced resistance faded much faster and more severely than the resistance induced in the NahG plants by BTH. Also seen in these experiments was that INA and BTH induced very good resistance in Ws to Emwa, and the *nim1-1*, *nim1-2* and other *nim1* alleles showed virtually no response to SA or INA with regard to disease resistance.

The *nim1* plants' lack of responsiveness to the SAR-inducing chemicals SA, INA, and BTH implies that the mutation is downstream of the entry point(s) for these chemicals in the signal transduction cascade leading to systemic acquired resistance.

Example 4: Northern Analysis of SAR Gene Expression

Since SA, INA and BTH did not induce SAR, or SAR gene expression in any of the *nim1* plants, it was of interest to investigate whether pathogen infection could induce SAR gene expression in these plants, as it does in wild type plants. Thus, the accumulation of SAR gene mRNA was also used as a criterion to characterize the different *nim1* alleles.

Wild-type seeds and seeds for each of the *nim1* alleles (*nim1-1, -2, -3, -4, -5, -6*) were sown onto MetroMix 300 growing media, covered with a transparent plastic dome, and placed at 4°C in the dark for 3 days. After 3 days of 4°C treatment, the plants were moved to a phytotron for 2 weeks. By approximately two weeks post-planting, germinated seedlings had produced 4 true leaves. Plants were then treated with H_2O, 5mM SA, 300 μM BTH ,or 300 μM INA. Chemicals were applied as a fine mist to completely cover the seedlings using a chromister. Water control plants were returned to the growing phytotron while the chemically treated plants were held in a separate but identical phytotron. At 3 days post-chemical application, water and chemically treated plants were inoculated with the compatible Emwa isolate. Noco inoculation was conducted on water treated plants only. Following inoculation, plants were covered with a clear plastic dome to maintain high humidity required for successful *P. parasitica* infection and placed in a growing chamber with 19°C day/17°C night temperatures and 8h light/16h dark cycles. RNA was extracted from plants 3 days after either water or chemical treatment, or 14 days after inoculation with

the compatible *P. parasitica* Emwa isolate. The RNA was size-fractionated by agarose gel electrophoresis and transferred to GeneScreen*Plus* membranes (DuPont).

Figures 1-3 present various RNA gel blots that indicate that SA, INA and BTH induce neither SAR nor SAR gene expression in *nim1* plants. In Figure 1, replicate blots were hybridized to *Arabidopsis* gene probes PR-1, PR-2 and PR-5 as described in Uknes *et al.* (1992). In contrast to the case in wild type plants, the chemicals did not induce RNA accumulation from any of these 3 SAR genes in *nim1-1* plants.

As shown in Figure 2, pathogen infection (Emwa) of wild type Ws-O plants induced PR-1 gene expression within 4 days after infection. In *nim1-1* plants, however, PR-1 gene expression was not induced until 6 days after infection and the level was reduced relative to the wild type at that time. Thus, following pathogen infection, PR-1 gene expression in *nim1-1* plants was delayed and reduced relative to the wild type.

The RNA gel blot in Figure 3 shows that *PR-1* mRNA accumulates to high levels following treatment of wild-type plants with SA, INA, or BTH or infection by *P. parasitica*. In the *nim1-1*, *nim1-2*, and *nim1-3* plants, *PR-1* mRNA accumulation was dramatically reduced relative to the wild type following chemical treatment. *PR-1* mRNA was also reduced following *P. parasitica* infection, but there was still some accumulation in these mutants. In the *nim1-4* and *nim1-6* plants, *PR-1* mRNA accumulation was more dramatically reduced than in the other alleles following chemical treatment (evident in longer exposures) and significantly less *PR-1* mRNA accumulated following *P. parasitica* infection, supporting the idea that these are particularly strong *nim1* alleles. *PR-1* mRNA accumulation was elevated in the *nim1-5* mutant, but only mildly induced following chemical treatment or *P. parasitica* infection. Based on both *PR-1* mRNA accumulation and fungal infection, the mutants have been determined to fall into three classes: severely compromised alleles (*nim1-4* and *nim1-6*); moderately compromised alleles (*nim1-1*, *nim1-2*, and *nim1-3*); and a weakly compromised allele (*nim1-5*).

Example 5: Determination of SA Accumulation in *nim1* Plants

Infection of wild type plants with pathogens that cause a necrotic reaction leads to accumulation of SA in the infected tissues. Endogenous SA is required for signal transduction in the SAR pathway, as breakdown of the endogenous SA leads to a decrease in disease resistance. This defines SA accumulation as a marker in the SAR pathway (Gaffney et al, 1993, Science 261, 754-756). The phenotype of *nim1* plants indicates a disruption in a component of the SAR pathway downstream of SA and upstream of SAR gene induction.

nim1 plants were tested for their ability to accumulate SA following pathogen infection. *Pseudomonas syringae* tomato strain DC 3000, carrying the avrRpt2 gene, was injected into leaves of 4-week-old *nim1* plants. The leaves were harvested 2 days later for SA analysis as described by Delaney et al, 1995, PNAS 92, 6602-6606. This analysis showed that the *nim1* plants accumulated high levels of SA in infected leaves, as shown in Figure 4. Uninfected leaves also accumulated SA, but not to the same levels as the infected leaves, similar to what has been observed in wild-type *Arabidopsis*. This indicates that the *nim* mutation maps downstream of the SA marker in the signal transduction pathway. Furthermore, INA and BTH (inactive in *nim1* plants) have been demonstrated to stimulate a component in the SAR pathway downstream of SA (Vernooij et al. (1995); Friedrich, et al. (1996); and Lawton, et al. (1996)). In addition, as described above, exogenously applied SA did not protect *nim1* plants from Emwa infection.

Example 6: Genetic Analysis

To determine dominance of the various mutants that display the *nim1* phenotype, pollen from wild type plants was transferred to the stigmata of *nim1-1, -2, -3, -4, -5, -6*. If the mutation is dominant, then the *nim1* phenotype will be observed in the resulting F1 plants. If the mutation is recessive, then the resulting F1 plants will exhibit a wild type phenotype.

The data presented in Table 3 show that when *nim1-1, -2, -3, -4* and *-6* were crossed with the wild type, the resulting F1 plants exhibited the wild type phenotype. Thus, these mutations are recessive. In contrast, the *nim1-5 X* wild type F1 descendants all exhibited the *nim1* phenotype, indicating that this is a dominant mutation. Following INA treatment, no *P. parasitica* sporulation was observed on wild type plants, while the F1 plants supported growth and some sporulation of *P. parasitica*. However, the *nim1* phenotype in these F1 plants was less severe than observed when *nim1-5* was homozygous.

To determine allelism, pollen from the kanamycin-resistant *nim1-1* mutant plants was transferred to the stigmata of *nim1-2*, -3, -4, -5, -6. Seeds resulting from the cross were plated onto Murashige-Skoog B5 plates containing kanamycin at 25 µg/ml to verify the hybrid origin of the seed. Kanamycin resistant (F1) plants were transferred to soil and assayed for the *nim1* phenotype. Because the F1 descendants of the cross of the *nim1-5* mutant with the Ws wild type display a *nim1* phenotype, analysis of *nim1-5 X nim1-1* F2 was also carried out.

As shown in Table 3, all of the resulting F1 plants exhibited the *nim1* phenotype. Thus, the mutation in the *nim1-2, -3, -4, -5, -6* was not complemented by the *nim1-1*; these

plants all fall within the same complementation group and are therefore allelic. F2 descendants from the *nim1-5 X nim1-1* cross also displayed the *nim1* phenotype, confirming that *nim1-5* is a *nim1* allele.

Table 3. Genetic Segregation of *nim* Mutants

Mutant	Generation	Female	Male	Phenotype: Wild type [a]	Phenotype: *nim1* [b]
nim1-1	F1	wild type [c]	*nim1-1*	24	0
	F2			98	32
nim1-2	F1	*nim1-2*	Wild type	3	0
nim1-3	F1	*nim1-3*	Wild type	3	0
nim1-4	F1	*nim1-4*	Wild type	3	0
nim1-5	F1	*nim1-5*	Wild type	0	35
	F1	*Wild type*	*nim1-5*	0	18
nim1-6	F1	*nim1-6*	Wild type	3	0
nim1-2	F1	*nim1-2*	*nim1-1*	0	15
nim1-3	F1	*nim1-3*	*nim1-1*	0	10
nim1-4	F1	*nim1-4*	*nim1-1*	0	15
nim1-5	F1	*nim1-5*	*nim1-1*	0	14
	F2			9	85
nim1-6	F1	*nim1-6*	*nim1-1*	0	12

[a] Number of plants with elevated PR-1 mRNA accumulation and absence of *P. parasitica* after INA treatment.
[b] Number of plants with no PR-1 mRNA accumulation and presence of *P. parasitica* after INA treatment.
[c] Wild type denotes the wild type Ws-0 strain.

B. Mapping of the *nim1* Mutation

Mapping of the *nim1* mutation is described in exhaustive detail in Applicants' U.S. Patent Application Serial No. 08/773,559, filed December 27, 1996, which is incorporated by reference herein in its entirety.

Example 7: Identification of Markers in and Genetic Mapping of the *NIM1* Locus

To determine a rough map position for *NIM1*, 74 F_2 *nim* plants from a cross between *nim1-1* (Ws-0) and Landsberg *erecta* (L*er*) were identified for their susceptibility to *P. parasitica* and lack of accumulation of *PR-1* mRNA following INA treatment. Using simple sequence length polymorphism (SSLP) markers (Bell and Ecker 1994), *nim1-1* was determined to lie about 8.2 centimorgans (cM) from nga128 and 8.2 cM from nga111 on the

lower arm of chromosome 1. In addition, *nim1-1* was determined to lie between nga111 and about 4 cM from the SSLP marker ATHGENEA. (Figure 5A)

For fine structure mapping, 1138 *nim* plants from an F_2 population derived from a cross between *nim1-1* and L*er* DP23 were identified based on both their inability to accumulate *PR-1* mRNA and their ability to support fungal growth following INA treatment. DNA was extracted from these plants and scored for zygosity at both ATHGENEA and nga111. As shown in Figure 5A, 93 recombinant chromosomes were identified between ATHGENEA and *nim1-1*, giving a genetic distance of approximately 4.1 cM (93 of 2276), and 239 recombinant chromosomes were identified between nga111 and *nim1-1*, indicating a genetic distance of about 10.5 cM (239 of 2276). Informative recombinants in the ATHGENEA to nga111 interval were further analyzed using amplified fragment length polymorphism (AFLP) analysis (Vos et al., 1995).

AFLP markers between ATHGENEA and nga111 were identified and were used to construct a low resolution map of the region (Figures 5A and 5B). AFLP markers W84.2 (1 cM from *nim1-1*) and W85.1 (0.6 cM from *nim1-1*) were used to isolate yeast artificial chromosome (YAC) clones from the CIC (for Centre d'Etude du Polymorphisme Humain, INRA and CNRS) library (Creusot et al., 1995). Two YAC clones, CIC12H07 and CIC12F04, were identified with W84.2 and two YAC clones CIC7E03 and CIC10G07 were identified with the W85.1 marker. (Figure 5B) To bridge the gap between the two sets of flanking YAC clones, bacterial artificial chromosome (BAC) and P1 clones that overlapped CIC12H07 and CIC12F04 were isolated and mapped, and sequential walking steps were carried out extending the BAC/P1 contig toward *NIM1* (Figure 5C; Liu et al., 1995; Chio et al., 1995). New AFLP's were developed during the walk that were specific for BAC or P1 clones, and these were used to determine whether the *NIM1* gene had been crossed. *NIM1* had been crossed when BAC and P1 clones were isolated that gave rise to both AFLP markers L84.6a and L84.8. The AFLP marker L84.6a found on P1 clones P1-18, P1-17, and P1-21 identified three recombinants and L84.8 found on P1 clones P1-20, P1-22, P1-23, and P1-24 and BAC clones, BAC-04, BAC-05, and BAC-06 identified one recombinant. Because these clones overlapped to form a large contig (>100 kb), and included AFLP markers that flanked *nim1*, the gene was determined to be located on the contig. The BAC and P1 clones that comprised the contig were used to generate additional AFLP markers, which showed that *nim1* was located between L84.Y1 and L84.8, representing a gap of about 0.09 cM.

C. Isolation of the *NIM1* Gene

Example 8: Construction of a Cosmid Contig

A cosmid library of the *NIM1* region was constructed in the *Agrobacterium*-compatible T-DNA cosmid vector pCLD04541 using CsCl-purified DNA from BAC-06, BAC-04, and P1-18. The DNAs of the three clones were mixed in equimolar quantities and were partially digested with the restriction enzyme Sau3A. The 20-25 kb fragments were isolated using a sucrose gradient, pooled and filled in with dATP and dGTP. Plasmid pCLD04541 was used as T-DNA cosmid vector. This plasmid contains a broad host range pRK290-based replicon, a tetracycline resistance gene for bacterial selection and the nptII gene for plant selection. The vector was cleaved with XhoI and filled in with dCTP and dTTP. The prepared fragments were then ligated into the vector . The ligation mix was packaged and transduced into *E. coli* strain XL1-blue MR (Stratagene). Resulting transformants were screened by hybridization with the BAC04, BAC06 and P1-18 clones and positive clones isolated. Cosmid DNA was isolated from these clones and template DNA was prepared using the ECs EcoRI/MseI and HindIII/MseI. The resulting AFLP fingerprint patterns were analyzed to determine the order of the cosmid clones. A set of 15 semi-overlapping cosmids was selected spanning the *nim* region (Figure 5D). The cosmid DNAs were also restricted with EcoRI, PstI, BssHII and SgrAI. This allowed for the estimation of the cosmid insert sizes and the verification of the overlaps between the various cosmids as determined by AFLP fingerprinting.

Physical mapping showed that the physical distance between L84.Y1 and L84.8 was >90 kb, giving a genetic to physical distance of ~1 megabase per cM. To facilitate the identification of the *NIM1* gene, the DNA sequence of BAC04 was determined.

Example 9: Identification of a Clone containing the *NIM1* Gene.

Cosmids generated from clones spanning the *NIM1* region were moved into *Agrobacterium tumefaciens* AGL-1 through conjugative transfer in a tri-parental mating with helper strain HB101 (pRK2013). These cosmids were then used to transform a kanamycin-sensitive *nim1-1* Arabidopsis line using vacuum infiltration (Bechtold et al., 1993; Mindrinos et al., 1994). Seed from the infiltrated plants was harvested and allowed to germinate on GM agar plates containing 50 mg/ml kanamycin as a selection agent. Only plantlets that were transformed with cosmid DNA could detoxify the selection agent and surviv . Seedlings that survived the selection were transferred to soil approximately two weeks after

plating and tested for the *nim1* phenotype as described below. Transformed plants that no longer had the *nim1* phenotype identified cosmid(s) containing a functional *NIM1* gene.

Example 10: Complementation of the *nim1* Phenotype

Plants transferred to soil were grown in a phytotron for approximately one week after transfer. 300µm INA was applied as a fine mist to completely cover the plants using a chromister. After two days, leaves were harvested for RNA extraction and PR-1 expression analysis. The plants were then sprayed with *Peronospora parasitica* (isolate Emwa) and grown under high humidity conditions in a growing chamber with 19°C day/17°C night temperatures and 8h light/16h dark cycles. Eight to ten days following fungal infection, plants were evaluated and scored positive or negative for fungal growth. Ws and *nim1* plants were treated in the same way to serve as controls for each experiment.

Total RNA was extracted from the collected tissue using a LiCl/phenol extraction buffer (Verwoerd, et al. 1989). RNA samples were run on a formaldehyde agarose gel and blotted to GeneScreen Plus (DuPont) membranes. Blots were hybridized with a ^{32}P-labeled PR-1 cDNA probe. The resulting blots were exposed to film to determine which transformants were able to induce PR-1 expression after INA treatment. The results are summarized in Table 4, which shows complementation of the *nim1* phenotype by cosmid clones D5, E1, and D7.

Table 4

Clone Name	# of transformants	# of plants with INA induced PR-1/ # of plants tested (%)
A8	3	0/3 (0%)
A11	8	4/18 (22%)
C2	10	1/10 (10%)
C7	33	1/32 (3%)
D2	81	4/49 (8%)
D5	6	5/6 (83%)
E1	10	10/10 (100%)
D7	129	36/36 (100%)
E8	9	0/9 (0%)
F12	6	0/6 (0%)
E6	1	0/1 (0%)
E7	34	0/4 (0%)
WS-control (wild-type)	NA	28/28 (100%)
nim1-1 phenotype control	NA	0/34 (0%)

NA - not applicable

Example 11: Sequencing of the *NIM1* Gene Region

BAC04 DNA (25 ug, obtained from KeyGene) was the source of DNA used for sequence analysis, as this BAC was the clone completely encompassing the region that complemented the *nim1* mutants. BAC04 DNA was randomly sheared in a nebulizer to generate fragments with an average length of about 2 kb. Ends of the sheared fragments were repaired, and the fragments were purified. Prepared DNA was ligated with EcoRV-digested pBRKanF4 (a derivative of $pBRKan_{F1}$ (Bhat 1993)). Resulting kanamycin-resistant colonies were selected for plasmid isolation using the Wizard Plus 9600 Miniprep System (Promega). Plasmids were sequenced using dye terminator chemistry (Applied BioSystems, Foster City, CA) with primers designed to sequence both strands of the plasmids (M13-21 forward and T7 reverse, Applied BioSystems). Data was collected on ABI377 DNA sequencers. Sequences were edited and assembled into contigs using Sequencher 3.0 (GeneCodes Corp., Ann Arbor, MI), the Staden genome assembly programs, phred, phrap and crossmatch (Phil Green, Washington University, St. Louis, MO) and consed (David Gordon, Washington University, St. Louis, MO). DNA from the cosmids found to complement the *nim1-1* mutation was sequenced using primers designed by Oligo 5.0 Primer Analysis Software (National Biosciences, Inc., Plymouth, MN). Sequencing of DNA from Ws-0 and the *nim1* alleles and cDNAs was performed essentially as described above.

A region of approximately 9.9 kb defined by the overlap of cosmids E1 and D7 was identified by complementation analysis to contain the *nim1* region. Primers that flanked the insertion site of the vector and that were specific to the cosmid backbone were designed using Oligo 5.0 Primer Analysis Software (National Biosciences, Inc.). DNA was isolated from cosmids D7 and E1 using a modification of the ammonium acetate method (Traynor, P.L., 1990. BioTechniques 9(6): 676.) This DNA was directly sequenced using Dye Terminator chemistry above. The sequence obtained allowed determination of the endpoints of the complementing region. The region defined by the overlap of cosmids E1 and D7 is presented as SEQ ID NO:1.

A truncated version of the BamHI-EcoRV fragment was also constructed, resulting in a construct that contained none of the "Gene 3" region (Fig. 5D). The following approach was necessary due the presence of HindIII sites in the Bam-Spe region of the DNA. The BamHI-EcoRV construct was completely digested with SpeI, then was split into two separate reactions for double digestion. One aliquot was digested with BamHI, the other HindIII. A BamHI-SpeI fragment of 2816 bp and a HindIII-SpeI fragment of 1588 bp were isolated from agarose gels (QiaQuick Gel extraction kit) and were ligated to BamHI-HindIII-

digested pSGCG01. DH5a was transformed with the ligation mix. Resulting colonies were screened for the correct insert by digestion with HindIII following preparation of DNA using Wizard Magic MiniPreps (Promega). A clone containing the correct construct was electroporated into *Agrobacterium* strain GV3101 for transformation of *Arabidopsis* plants.

Example 12: Sequence Analysis and Subcloning of the *NIM1* Region

The 9.9 kb region containing the *NIM1* gene was analyzed for the presence of open reading frames in all six frames using Sequencher 3.0 and the GCG package. Four regions containing large ORF's were identified as possible genes (Gene Regions 1-4 in Figure 5D). These four regions were PCR amplified from DNA of the wild-type parent and the six different *nim1* allelic variants *nim1-1, -2, -3, -4, -5,* and *-6.* Primers for these amplifications were selected using Oligo 5.0 (National Biosciences, Inc.) and were synthesized by Integrated DNA Technologies, Inc. PCR products were separated on 1.0% agarose gels and were purified using the QIAquick Gel Extraction Kit. The purified genomic PCR products were directly sequenced using the primers used for the initial amplification and with additional primers designed to sequence across any regions not covered by the initial primers. Average coverage for these gene regions was approximately 3.5 reads/base.

Sequences were edited and were assembled using Sequencher 3.0. Base changes specific to various *nim1* alleles were identified only in the region designated Gene Region 2, as shown below in Table 5, which shows sequence variations among all six of the *nim1* alleles.

Table 5

	Gene Region			
Allele/ ecotype	1 (bases 590-1090)	2 (*NIM1*) (bases 1380-4100)	3 (bases 5870 - 6840)	4 (bases 8140-9210)
nim1-1	no changes	t inserted at 2981: change of 7AA and premature termination of protein.	no changes	no changes
nim1-2	no changes	g to a at 2799: His to Tyr	no changes	no changes
nim1-3	no changes	deletion of t at 3261: change of 10AA and premature termination of protein.	no changes	no changes
nim1-4	no changes	c to t at 2402: Arg to lys	no changes	no changes
nim1-5	no changes	c to t at 2402: Arg to lys	no changes	no changes
nim1-6	g to a at 734: asp to lys	g to a at 2670: Gln to Stop	no changes	no changes
WS (compared to Columbia)	no changes	a to g at 1607: Ile to Leu a to c at 2344: intron t to g at 2480: Gln to Pro g to c at 2894: Ser to Trp ggc deleted at 3449: lose Ala c to t at 3490: Ala to Thr c to t at 3498: Ser to Asn a to t at 3873: non-coding g to a at 3992: non-coding g to a at 4026: non-coding g to a at 4061: non-coding	t to a at 5746 a to t at 5751 t to a at 5754 c to t at 6728 a to t at 6815 t to c at 6816	t to g at 8705 g to t at 8729 g to t at 8739 g to t at 8784 c to a at 8789 c to t at 8812 a to g at 8829 t to g at 8856 a to c at 9004 a to t at 9011 a to g at 8461
RNA detected	No	Yes	No	No

Positions listed in the table relate to SEQ ID NO:1. All alleles *nim1-1* to *nim1-6* are WS strain. Columbia-0 represents the wild type

It is apparent that the *NIM1* gene lies within Gene Region 2, because there are amino acid changes or alterations of sequence within the open reading frame of Gene Region 2 in all six *nim1* alleles. At the same time, at least one of the *nim1* alleles shows no changes in the open reading frames within Gene Regions 1, 3 and 4. Therefore, the only gene region within the 9.9 kb region that could contain the *NIM1* gene is Gene Region 2.

The Ws section of Table 5 indicates the changes in the Ws ecotype of *Arabidopsis* relative to the Columbia ecotype of *Arabidopsis*. The sequences presented herein relate to the Columbia ecotype of *Arabidopsis*, which contains the wild type gene in the experiments described herein. The changes are listed as amino acid changes within Gene Region 2 (the *NIM1* region) and are listed as changes in base pairs in the other regions.

The cosmid region containing the *nim1* gene was delineated by a BamH1-EcoRV restriction fragment of ~5.3 kb. Cosmid DNA from D7 and plasmid DNA from pBlueScriptII(pBSII) were digested with BamHI and with EcoRV (NEB). The 5.3 kb fragment from D7 was isolated from agarose gels and was purified using the QIAquick gel extraction kit (# 28796, Qiagen). The fragment was ligated overnight to the Bam-EcoRV-digested pBSII and the ligation mixture was transformed into *E. coli* strain DH5a. Colonies containing the insert were selected, DNA was isolated, and confirmation was made by digestion with HindIII. The Bam-EcoRV fragment was then engineered into a binary vector (pSGCG01) for transformation into *Arabidopsis*.

Example 13: Northern Analysis of the Four Gene Regions

Identical Northern blots were made from RNA samples isolated from water-, SA-, BTH- and INA-treated Ws and *nim1* lines as previously described in Delaney, et al. (1995). These blots were hybridized with PCR products generated from the four gene regions identified in the 9.9 kb *NIM1* gene region (SEQ ID NO:1). Only the gene region containing the *NIM1* gene (Gene Region 2) had detectable hybridization with the RNA samples, indicating that only the *NIM1* region contains a detectable transcribed gene (Figure 5D and Table 5).

Example 14: Complementation with Gene Region 2

Gene Region 2 (Fig. 5D) was also demonstrated to contain the functional *NIM1* gene by doing additional complementation experiments. A BamHI/HindIII genomic DNA fragment containing Gene Region 2 was isolated from cosmid D7 and was cloned into the binary vector pSGCG01 containing the gene for kanamycin resistance. The resulting plasmid was transformed into the Agrobacterium strain GV3101 and positive colonies were selected on kanamycin. PCR was used to verify that the selected colony contains the plasmid. Kanamycin-sensitive *nim1-1* plants were infiltrated with this bacteria as previously described. The resulting seed was harvested and planted on GM agar containing 50µg/ml kanamycin. Plants surviving selection were transferred to soil and tested for complementation. Transformed plants and control Ws and *nim1* plants were sprayed with 300µm INA. Two days later, leaves were harvested for RNA extraction and PR-1 expression analysis. The plants were then sprayed with *Peronospora parasitica* (isolate Emwa) and grown as previously described. Ten days following fungal infection, plants were

evaluated and scored positive or negative for fungal growth. All of the 15 transformed plants, as well as the Ws controls, were negative for fungal growth following INA treatment, while the *nim1* controls were positive for fungal growth. RNA was extracted and analyzed as described above for these transformants and controls. Ws controls and all 15 transformants showed PR-1 gene induction following INA treatment, while the *nim1* controls did not show PR-1 induction by INA.

Example 15: Isolation of a *NIM1* cDNA

An Arabidopsis cDNA library made in the lYES expression vector (Elledge et al, 1991, PNAS 88, 1731-1735) was plated and plaque lifts were performed. Filters were hybridized with a ^{32}P-labeled PCR product generated from Gene Region 2 (Figure 5D). 14 positives were identified from a screen of approximately 150,000 plaques. Each plaque was purified and plasmid DNA was recovered. cDNA inserts were digested out of the vector using EcoRI, agarose-gel-purified and sequenced. Sequence obtained from the longest cDNA is indicated in SEQ ID NO:2 and Figure 6. To confirm that the 5' end of the cDNA had been obtained, a Gibco BRL 5' RACE kit was used following manufacturer's instructions. The resulting RACE products were sequenced and found to include the additional bases indicated in Figure 6. The transcribed region present in both cDNA clones and detected in RACE is shown as capital letters in Figure 6. Changes in the alleles are shown above the DNA strand. Capitals indicate the presence of the sequence in a cDNA clone or detected after RACE PCR.

The same RNA samples produced in the induction studies (Figure 3) were also probed with the *NIM1* gene using a full-length cDNA clone as a probe. In Figure 7 it can be seen that INA induced the *NIM1* gene in the wild type Ws allele. However, the *nim1-1* mutation allele showed a lower basal level expression of the *NIM1* gene, and it was not inducible by INA. This was similar to what was observed in the *nim1-3* allele and the *nim1-6* allele. The *nim1-2* allele showed approximately normal levels in the untreated sample and showed similar induction to that of the wild type sample, as did the *nim1-4* allele. The *nim1-5* allele seemed to show higher basal level expression of the *NIM1* gene and much stronger expression when induced by chemical inducers.

D. *NIM1* Homologues

Example 16: BLAST Search with the *NIM1* Sequence

A multiple sequence alignment was constructed using Clustal V (Higgins, Desmond G. and Paul M. Sharp (1989), Fast and sensitive multiple sequence alignments on a microcomputer, CABIOS 5:151-153) as part of the DNA* (1228 South Park Street, Madison Wisconsin, 53715) Lasergene Biocomputing Software package for the Macintosh (1994). Certain regions of the *NIM1* protein are homologous in amino acid sequence to 4 different rice cDNA protein products. The homologies were identified using the *NIM1* sequences in a GenBank BLAST search. Comparisons of the regions of homology in *NIM1* and the rice cDNA products are shown in Figure 8 (*See also*, SEQ ID NO:3 and SEQ ID NO's: 4-11). The *NIM1* protein fragments show from 36 to 48% identical amino acid sequences with the 4 rice products.

Example 17: Isolation of Homologous Genes from Other Plants

Using the *NIM1* cDNA as a probe, homologs of Arabidopsis *NIM1* are identified through screening genomic or cDNA libraries from different crops such as, but not limited to those listed below in Example 22. Standard techniques for accomplishing this include hybridization screening of plated DNA libraries (either plaques or colonies; see, *e.g.* Sambrook *et al.*, Molecular Cloning , eds., Cold Spring Harbor Laboratory Press. (1989)) and amplification by PCR using oligonucleotide primers (see, *e.g.* Innis *et al.*, PCR Protocols, a Guide to Methods and Applications eds., Academic Press (1990)). Homologs identified are genetically engineered into the expression vectors herein and transformed into the above listed crops. Transformants are evaluated for enhanced disease resistance using relevant pathogens of the crop plant being tested.

NIM1 homologs in the genomes of cucumber, tomato, tobacco, maize, wheat and barley have been detected by DNA blot analysis. Genomic DNA was isolated from cucumber, tomato, tobacco, maize, wheat and barley, restriction digested with the enzymes BamHI, HindIII, XbaI, or SalI, electrophoretically separated on 0.8% agarose gels and transferred to nylon membrane by capillary blotting. Following UV-crosslinking to affix the DNA, the membrane was hybridized under low stringency conditions [(1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride) at 55°C for 18-24h] with ^{32}P-radiolabelled *Arabidopsis thaliana* NIM1 cDNA. Following hybridization the blots were washed under low stringency conditions [6XSSC for 15 min.

(X3) 3XSSC for 15 min. (X1) at 55°C; 1XSSC is 0.15M NaCl, 15mM Na-citrate (pH7.0)] and exposed to X-ray film to visualize bands that correspond to *NIM1*.

In addition, expressed sequence tags (EST) identified with similarity to the *NIM1* gene such as the rice EST's described in Example 16 can also be used to isolate homologues. The rice EST's may be especially useful for isolation of *NIM1* homologues from other monocots.

Homologues may be obtained by PCR. In this method, comparisons are made between known homologues (e.g., rice and Arabidopsis). Regions of high amino acid and DNA similarity or identity are then used to make PCR primers. Regions rich in M and W are best followed by regions rich in F, Y, C, H, Q, K and E because these amino acids are encoded by a limited number of codons. Once a suitable region is identified, primers for that region are made with a diversity of substitutions in the 3rd codon position. This diversity of substitution in the third position may be constrained depending on the species that is being targeted. For example, because maize is GC rich, primers are designed that utilize a G or a C in the 3rd position, if possible.

The PCR reaction is performed from cDNA or genomic DNA under a variety of standard conditions. When a band is apparent, it is cloned and/or sequenced to determine if it is a *NIM1* homologue.

E. Overexpression of *NIM1* Confers Disease Resistance In Plants

Overexpression of the *NIM1* gene in transgenic plants to confer a CIM phenotype is also described in Applicants' U.S. Patent Application Serial No. 08/773,554, filed December 27, 1996, which is incorporated by reference herein in its entirety.

Example 18: Overexpression Expression of *NIM1* Due To Insertion Site Effect

To determine if any of the transformants described above in Example 10/Table 4 had overexpression of *NIM1* due to insertion site effect, primary transformants containing the D7, D5 or E1 cosmids (containing the *NIM1* gene) were selfed and the T2 seed collected. Seeds from one E1 line, four D5 lines and 95 D7 lines were sown on soil and grown as described above. When the T2 plants had obtained at least four true leaves, a single leaf was harvested separately for each plant. RNA was extracted from this tissue and analyzed for PR-1 and *NIM1* expression. Plants were then inoculated with *P. parasitica* (Emwa) and analyzed for fungal growth at 3-14 days, preferably 7-12 days, following infection. Plants

showing higher than normal *NIM1* and PR-1 expression and displaying fungal resistance demonstrated that overexpression of *NIM1* confers a CIM phenotype.

Table 6 shows the results of testing of various transformants for resistance to fungal infection. As can be seen from the table, a number of transformants showed less than normal fungal growth and several showed no visible fungal growth at all. RNA was prepared from collected samples and analyzed as previously described (Delaney et al, 1995). Blots were hybridized to the *Arabidopsis* gene probe PR-1 (Uknes et al, 1992). Lines D7-74, D5-6 and E1-1 showed early induction of PR-1 gene expression, whereby PR-1 mRNA was evident by 24 or 48 hours following fungal treatment. These three lines also demonstrated resistance to fungal infection.

Table 6

Line	P.parasitica growth	Line	P.parasitica growth	Line	P.parasitica growth
D7-2	negative	52	+	90	+
3	+	53	+	91	+
9	+	54	+/-	92	+
11	+	56	+	93	+
12	+	57	+	94	+
13	+	58	+	95	+
14	+	59	+	96	+
17	+	60	+	97	+
18	+	61	+	98	+/-
19	+	62	+	100	+/-
20	+	63	+	101	+/-
21	+	64	+	102	+/-
22	+	66	+	103	+
23	+	67	+	104	+
24	+	68	+	106	+
25	+	69	+	107	+
28	+	70	+	108	+
29	+	71	+	114	+
31	+	72	+	115	+
32	+	73	+	118	+
33	+	74	negative	119	+
34	+	75	+	122	+
35	+	77	+	123	+
36	+	78	+	124	+
38	+	79	+	125	+
39	+	80	+/-	126	+
42	+	81	+	128	+
43	+	82	+	129	+
46	+	83	+	130	+

47	+	84	+	D5-1	+
48	+	85	+	2	+
49	+	86	+	4	+
50	+	87	+/-	6	+/-
51	+	89	negative	E1-1	negative

Plants were treated with P. parasitica isolate Emwa and scored 10 days later.
+, normal fungal growth
+/-, less than normal fungal growth
negative, no visible fungal growth

Example 19: *NIM1* Overexpression Under Its Native Promoter

Plants constitutively expressing the *NIM1* gene were generated from transformation of Ws wild type plants with the *BamHI-HindIII NIM1* genomic fragment (SEQ ID NO: 2 - bases 1249-5655) containing 1.4 kb of promoter sequence. This fragment was cloned into pSGCG01 and transformed into the Agrobacterium strain GV3101 (pMP90, Koncz and Schell (1986) *Mol. Gen. Genet.* 204:383-396). Ws plants were infiltrated as previously described. The resulting seed was harvested and plated on GM agar containing 50 µg/ml kanamycin. Surviving plantlets were transferred to soil and tested as described above for resistance to *Peronospora parasitica* isolate Emwa. Selected plants were selfed and selected for two subsequent generations to generate homozygous lines. Seeds from several of these lines were sown in soil and 15-18 plants per line were grown for three weeks and tested again for Emwa resistance without any prior treatment with an inducing chemical. Approximately 24 hours, 48 hours, and five days after fungal treatment, tissue was harvested, pooled and frozen for each line. Plants remained in the growth chamber until ten days after inoculation when they were scored for resistance to Emwa.

RNA was prepared from all of the collected samples and analyzed as previously described (Delaney et al, 1995). The blot was hybridized to the *Arabidopsis* gene probe PR-1 (Uknes et al, 1992). Five of the 13 transgenic lines analyzed showed early induction of PR1 gene expression. For these lines, PR-1 mRNA was evident by 24 or 48 hours following fungal treatment. These five lines also had no visible fungal growth. Leaves were stained with lactophenol blue as described (Dietrich et al., 1994) to verify the absence of fungal hyphae in the leaves. PR-1 gene expression was not induced in the other eight lines by 48 hours and these plants did not show resistance to Emwa.

A subset of the resistant lines were also tested for increased resistance to the bacterial pathogen *Pseudomonas syringae* DC3000 to evaluate the spectrum of resistance evident as described by Uknes et al. (1993). Experiments were done essentially as described by Lawton et al. (1996). Bacterial growth was slower in those lines that also

demonstrated constitutive resistance to Emwa. This shows that plants overexpressing the *NIM1* gene under its native promoter have constitutive immunity against pathogens.

To assess additional characteristics of the CIM phenotype in these lines, unifected plants are evaluated for free and glucose-conjugated salicylic acid and leaves are stained with lactophenol blue to evaluate for the presence of microscopic lesions. Resistance plants are sexually crossed with SAR mutants such as NahG and *ndr1* to establish the epistatic relationship of the resistance phenotype to other mutants and evaluate how these dominant negative mutants of *NIM1* may influence the salicylic acid-dependent feedback loop.

Example 20: 35S Driven Overxpression of *NIM1*

The full-length *NIM1* cDNA (SEQ ID NO: 21) was cloned into the *EcoRI* site of pCGN1761 ENX (Comai et al. (1990) *Plant Mol. Biol.* 15, 373-381). From the resulting plasmid, an *XbaI* fragment containing an enhanced CaMV 35S promoter, the *NIM1* cDNA in the correct orientation for transcription, and a tml 3' terminator was obtained. This fragment was cloned into the binary vector pCIB200 and transformed into GV3101. Ws plants were infiltrated as previously described. The resulting seed was harvested and plated on GM agar containing 50 µg/ml kanamycin. Surviving plantlets were transferred to soil and tested as described above. Selected plants were selfed and selected for two subsequent generations to generate homozygous lines. Nine of the 58 lines tested demonstrated resistance when they were treated with Emwa without prior chemical treatment. Thus, overexpression of the *NIM1* cDNA also results in disease-resistant plants.

Example 21: High Level Expression of *NIM1* in Crop Plants

Those constructs conferring a CIM phenotype in Col-0 or Ws-0 and others are transformed into crop plants for evaluation. Although the *NIM1* gene can be inserted into any plant cell falling within these broad classes, it is particularly useful in crop plant cells, such as rice, wheat, barley, rye, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane. Transformants are evaluated for enhanced disease resistance. In a preferred embodiment of the invention, the expression of the *NIM1* gene is at a lev l

which is at least two-fold above the expression level of the *NIM1* gene in wild type plants and is preferably ten-fold above the wild type expression level.

F. Other Uses of *nim* Phenotype Plants Generally

Example 22: The Use of *nim* Mutants in Disease Testing

nim mutants are challenged with numerous pathogens and found to develop larger lesions more quickly than wild-type plants. This phenotype is referred to as UDS (i.e. universal disease susceptibility) and is a result of the mutants failing to express SAR genes to effect the plant defense against pathogens. The UDS phenotype of *nim* mutants renders them useful as control plants for the evaluation of disease symptoms in experimental lines in field pathogenesis tests where the natural resistance phenotype of so-called wild type lines may vary (i.e. to different pathogens and different pathotypes of the same pathogen). Thus, in a field environment where natural infection by pathogens is being relied upon to assess the resistance of experimental lines, the incorporation into the experiment of *nim* mutant lines of the appropriate crop plant species would enable an assessment of the true level and spectrum of pathogen pressure, without the variation inherent in the use of non-experimental lines.

Example 23: Assessment of the Utility of Transgenes for the Purposes of Disease Resistance

nim mutants are used as host plants for the transformation of transgenes to facilitate their assessment for use in disease resistance. For example, an Arabidopsis *nim* mutant line, characterized by its UDS phenotype, is used for subsequent transformations with candidate genes for disease resistance thus enabling an assessment of the contribution of an individual gene to resistance against the basal level of the UDS *nim* mutant plants.

Example 24: *nim* Mutants as a Tool in Understanding Plant-Pathogen Interactions

nim mutants are useful for the understanding of plant pathogen interactions, and in particular for the understanding of the processes utilized by the pathogen for the invasion of plant cells. This is so because *nim* mutants do not mount a systemic response to pathogen attack, and the unabated development of the pathogen is an ideal scenario in which to study its biological interaction with the host.

Of futher significance is the observation that a host *nim* mutant may be susceptible to pathogens not normally associated with that particular host, but instead associated with a different host. For example, an Arabidopsis *nim* mutant such as *nim1-1, -2, -3, -4, -5, or -6* is challenged with a number of pathogens that normally only infect tobacco, and found to be susceptible. Thus, the *nim* mutation causing the UDS phenotype leads to a modification of pathogen-range susceptibility and this has significant utility in the molecular, genetic and biochemical analysis of host-pathogen interaction.

Example 25: *nim* Mutants for Use in Fungicide Screening

nim mutants are particularly useful in the screening of new chemical compounds for fungicide activity. *nim* mutants selected in a particular host have considerable utility for the screening of fungicides using that host and pathogens of the host. The advantage lies in the UDS phenoytpe of the mutant that circumvents the problems encountered by the host being differentially susceptible to different pathogens and pathotypes, or even resistant to some pathogens or pathotypes. By way of example, *nim* mutants in wheat could be effectively used to screen for fungicides to a wide range of wheat pathogens and pathotypes as the mutants would not mount a resistance response to the introduced pathogen and would not display differential resistance to different pathotypes that might otherwise require the use of multiple wheat lines, each adequately susceptible to a particular test pathogen. Wheat pathogens of particular interest include (but are not limited to) *Erisyphe graminis* (the causative agent of powdery mildew), *Rhizoctonia solani* (the causative agent of sharp eyespot), *Pseudocercosporella herpotrichoides* (the causative agent of eyespot), *Puccinia spp.* (the causative agents of rusts), and *Septoria nodorum*. Similarly, *nim* mutants of corn would be highly susceptible to corn pathogens and therefore useful in the screening for fungicides with activity against corn diseases.

nim mutants have further utility for the screening of a wide range of pathogens and pathotypes in a heterologous host i.e. in a host that may not normally be within the host species range of a particular pathogen and that may be particularly easily to manipulate (such as Arabidopsis). By virtue of its UDS phenotype the heterologous host is susceptible to pathogens of other plant species, including economically important crop plant species. Thus, by way of example, the same Arabidopsis *nim* mutant could be infected with a wheat pathogen such as *Erisyphe graminis* (the causative agent of powdery mildew) or a corn pathogen such as *Helminthosporium maydis* and used to test the efficacy of fungicide candidates. Such an approach has considerable improvements in efficiency over currently used procedures of screening individual crop plant species and different cultivars of species

with different pathogens and pathotypes that may be differentially virulent on the different crop plant cultivars. Furthermore, the use of Arabidopsis has advantages because of its small size and the possibility of thereby undertaking more tests with limited resources of space.

Example 26: NIM1 Is A Homolog Of IκBα

A multiple sequence alignment between the protein gene products of *NIM1* and IkB was performed by which it was determined that the *NIM1* gene product is a homolog of IκB α (Figure 9). Sequence homology searches were performed using BLAST (Altschul *et al., J. Mol. Biol.* 215, 403-410 (1990)). The multiple sequence alignment was constructed using Clustal V (Higgins et al., *CABIOS* 5,151-153 (1989)) as part of the Lasergene Biocomputing Software package from DNASTAR (Madison, WI). The sequences used in the alignment were NIM1 (SEQ ID NO:3), mouse IκBα (SEQ ID NO:18, GenBank Accession #: 1022734), rat IκBα (SEQ ID NO:19, GenBank accession Nos. 57674 and X63594; Tewari et al., *Nucleic Acids Res.* 20, 607 (1992)), and pig IκBα (SEQ ID NO:20, GenBank accession No. Z21968; de Martin et al., *EMBO J.* 12, 2773-2779 (1993); GenBank accession No. 517193, de Martin et al., *Gene* 152, 253-255 (1995)). Parameters used in the Clustal analysis were gap penalty of 10 and gap length penalty of 10. Evolutionary divergence distances were calculated using the PAM250 weight table (Dayhoff et al., "A model of evolutionary change in proteins. Matrices for detecting distant relationships." In *Atlas of Protein Sequence and Structure*, Vol. 5, Suppl. 3, M.O., Dayhoff, ed (National Biomedical Research Foundation, Washington, D.C.), pp. 345-358 (1978)). Residue similarity was calculated using a modified Dayhoff table (Schwartz and Dayhoff, "A model of evolutionary change in proteins." In *Atlas of Protein Sequence and Structure*, M.O. Dayhoff, ed (National Biomedical Research Foundation, Washington, D.C.) pp. 353-358 (1979); Gribskov and Burgess, *Nucleic Acids Res.* 14, 6745-6763 (1986)).

Homology searches indicate similarity of NIM1 to ankyrin domains of several proteins including: ankyrin, NF-κB and IκB. The best overall homology is to IκB and related molecules (Figure 9). NIM1 contains 2 serines at amino acid positions 55 and 59, the serine at position 59 is in a context (D/ExxxxxS) and position (N-terminal) consistent with a role in phosphorylation-dependent, ubiquitin-mediated, inducible degradation. All IκBs have these N-terminal serines and they are required for inactivation of IκB and subsequent release of NF-κB. NIM1 has ankyrin domains (amino acids 262-290 and 323-371). Ankyrin domains are believed to be involved in protein-protein interactions and are a ubiquitous feature for IκB and NF-κB molecules. The C-termini of IκB's can be dissimilar. NIM1 has

some homology to a QL-rich region (amino acids 491-499) found in the C-termini of some IκBs.

Example 27: Generation Of Altered Forms Of *NIM1* - Changes Of Serine Residues 55 and 59 To Alanine Residues

Phosphorylation of serine residues in human IκBα is required for stimulus-activated degradation of IκBα thereby activating NF-κB. Mutagenesis of the serine residues (S32-S36) in human IκBα to alanine residues inhibits stimulus-induced phosphorylation thus blocking IκBα proteosome-mediated degradation (E. Britta-Mareen Traenckner et al., *EMBO J.* 14: 2876-2883 (1995); Brown et al., *Science* 267:1485-1488 (1996); Brockman et al., *Molecular and Cellular Biology* 15: 2809-2818 (1995); Wang et al., *Science* 274:784-787 (1996)).

This altered form of IκBα functions as a dominant negative form by retaining NF-κB in the cytoplasm, thereby blocking downstream signaling events. Based on sequence comparisons between NIM1 and IκB, serines 55 (S55) and 59 (S59) of NIM1 are homologous to S32 and S36 in human IκBα. To construct dominant-negative forms of NIM1, the serines at amino acid positions 55 and 59 are mutagenized to alanine residues. This can be done by any method known to those skilled in the art, such as, for example, by using the QuikChange Site Directed Mutagenesis Kit (#200518:Strategene).

Using a full length *NIM1* cDNA (SEQ ID NO:21) including 42 bp of 5' untranslated sequence (UTR) and 187 bp of 3' UTR, the mutagenized construct can be made per the manufacturer's instructions using the following primers (SEQ ID NO:21, positions 192-226): 5'-CAA CAG CTT CGA AGC CGT CTT TGA CGC GCC GGA TG-3' (SEQ ID NO:32) and 5'-CAT CCG GCG CGT CAA AGA CGG CTT CGA AGC TGT TG-3' (SEQ ID NO:33), where the underlined bases denote the mutations. The strategy is as follows: The *NIM1* cDNA cloned into vector pSE936 (Elledge et al., *Proc. Nat. Acad. Sci. USA* 88:1731-1735 (1991)) is denatured and the primers containing the altered bases are annealed. DNA polymerase (Pfu) extends the primers by nonstrand-displacement resulting in nicked circular strands. DNA is subjected to restriction endonuclease digestion with DpnI, which only cuts methylated sites (nonmutagenized template DNA). The remaining circular dsDNA is transformed into *E.coli* strain XL1-Blue. Plasmids from resulting colonies are extracted and sequenced to verify the presence of the mutated bases and to confirm that no other mutations occurred.

The mutagenized *NIM1* cDNA is digested with the restriction endonuclease EcoRI and cloned into pCGN1761 under the transcriptional regulation of the double 35S promoter of

the cauliflower mosaic virus. The transformation cassette including the 35S promoter, *NIM1* cDNA and *tml* terminator is released from pCGN1761 by partial restriction digestion with XbaI and ligated into the XbaI and ligated into the XbaI site of dephosphorylated pCIB200. SEQ ID NO's:22 and 23 show the DNA coding sequence and encoded amino acid sequence, respectively, of this altered form of the *NIM1* gene.

The present invention also encompasses altered forms of alleles of *NIM1*, wherein the coding sequence of such an allele hybridizes under the following conditions to the coding sequence set forth in SEQ ID NO:22: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C. In these embodiments, alleles of *NIM1* hybridizing to SEQ ID NO: 22 under these conditions are altered so that the encoded product has alanines instead of serines in the amino acid positions that correspond to positions 55 and 59 of SEQ ID NO: 22.

Example 28: Generation Of Altered Forms Of *NIM1* - N-terminal Deletion

Deletion of amino acids 1-36 (Brockman et al.; Sun et al.) or 1-72 (Sun et al.) of human IκBα, which includes K21, K22, S32 and S36, results in a dominant-negative IκBα phenotype in transfected human cell cultures. An N-terminal deletion of approximately the first 125 amino acids of the encoded product of the *NIM1* cDNA removes eight lysine residues that may serve as potential ubiquitination sites and also removes putative phosphorylation sites at S55 and S59 (see Example 2). This altered gene construct may be produced by any means known to those skilled in the art. For example, using the method of Ho *et al.*, *Gene* 77:5l-59 (l989), a *NIM1* form may be generated in which DNA encoding approximately the first 125 amino acids is deleted. The following primers produce a 1612-bp PCR product (SEQ ID NO:21: 418 to 2011): 5'-gg aat tca-ATG GAT TCG GTT GTG ACT GTT TTG-3' (SEQ ID NO:34) and 5'-gga att cTA CAA ATC TGT ATA CCA TTG G-3' (SEQ ID NO:35) in which the synthetic start codon is underlined (ATG) and EcoRI linker sequence is in lower case. Amplification of fragments utilizes a reaction mixture comprising 0.1 to 100 ng of template DNA, 10mM Tris pH 8.3/50mM KCl/2 mM $MgCl_2$/0.001% gelatin/0.25 mM each dNTP/0.2 mM of each primer and 1 unit rTth DNA polymerase in a final volume of 50 mL and a Perkin Elmer Cetus 9600 PCR machine. PCR conditions are as follows: 94°C 3min: 35x (94°C 30 sec: 52°C 1 min: 72°C 2 min): 72°C 10 min. The PCR product is cloned directly into the pCR2.1 vector (Invitrogen). The PCR-generated ins rt in the PCR vector is released by restriction endonuclease digestion using *EcoRI* and ligat d into the *EcoRI* site of dephosphorylated pCGN1761, under the transcriptional regulation of the double 35S

promoter. The construct is sequenced to verify the presence of the synthetic starting ATG and to confirm that no other mutations occurred during PCR. The transformation cassette including the 35S promoter, modified *NIM1* cDNA and *tml* terminator is released from pCGN1761ENX by partial restriction digestion with XbaI and ligated into the XbaI site of pCIB200. SEQ ID NO's:24 and 25 show the DNA coding sequence and encoded amino acid sequence, respectively, of an altered form of the *NIM1* gene having an N-terminal amino acid deletion.

The present invention also encompasses altered forms of alleles of *NIM1*, wherein the coding sequence of such an allele hybridizes under the following conditions to the coding sequence set forth in SEQ ID NO:24: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C. In these embodiments, alleles of *NIM1* hybridizing to SEQ ID NO:24 under these conditions are altered so that the encoded product has an N-terminal deletion that removes lysine residues that may serve as potential ubiquitination sites in addition to the serines at amino acid positions corresponding to positions 55 and 59 of the wild-type gene product.

Example 29: Generation Of Altered Forms Of *NIM1* - C-terminal Deletion

The deletion of amino acids 261-317 of human IκBα is believed to result in enhanced intrinsic stability by blocking the constitutive phosphorylation of serine and threonine residues in the C-terminus. A region rich in serine and threonine is present at amino acids 522-593 in the C-terminus of NIM1. The C-terminal coding region of the *NIM1* gene may be modified by deleting the nucleotide sequences which encode amino acids 522-593. Using the method of Ho et al. (I989), the C-terminal coding region and 3' UTR of the *NIM1* cDNA (SEQ ID NO:21: 1606-2011) is deleted by PCR, generating a 1623 bp fragment using the following primers: 5'-cggaattcGATCTCTTTAATTTGTGAATTT C-3' (SEQ ID NO:36) and 5'-ggaattcTCAACAGTT CATAATCTGGTCG-3' (SEQ ID NO:37) in which a synthetic stop codon is underlined (TGA on complementary strand) and *EcoRI* linker sequences are in lower case. PCR reaction components are as previously described and cycling parameters are as follows: 94°C 3 min: 30x (94°C 30 sec: 52°C 1 min: 72°C 2 min); 72°C 10 min]. The PCR product is cloned directly into the pCR2.1 vector (Invitrogen). The PCR-generated insert in the PCR vector is released by restriction endonuclease digestion using *EcoRI* and ligated into the *EcoRI* site of dephosphorylated pCGN1761, which contains the double 35S promoter. The construct is sequenced to verify the presence of the synthetic in-frame stop codon and to confirm that no other mutations occurred during

PCR. The transformation cassette including the promoter, modified *NIM1* cDNA, and *tml* terminator is released from pCGN1761 by partial restriction digestion with *XbaI* and ligated into the *XbaI* site of dephosphorylated pCIB200. SEQ ID NO's:26 and 27 show the DNA coding sequence and encoded amino acid sequence, respectively, of an altered form of the *NIM1* gene having a C-terminal amino acid deletion.

The present invention also encompasses altered forms of alleles of *NIM1*, wherein the coding sequence of such an allele hybridizes under the following conditions to the coding sequence set forth in SEQ ID NO:26: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C. In these embodiments, alleles of *NIM1* hybridizing to SEQ ID NO:26 under the above conditions are altered so that the encoded product has a C-terminal deletion that removes serine and threonine residues.

Example 30: Generation Of Altered Forms Of *NIM1* - N-terminal/C-terminal Deletion Chimera

An N-terminal and C-terminal deletion form of *NIM1* is generated using a unique *KpnI* restriction site at position 819 (SEQ ID NO:21). The N-terminal deletion form (Example 28) is restriction endonuclease digested with *EcoRI/KpnI* and the 415 bp fragment corresponding to the modified N-terminus is recovered by gel electrophoresis. Likewise, the C-terminal deletion form (Example 29) is restriction endonuclease digested with *EcoRI/KpnI* and the 790 bp fragment corresponding to the modified C-terminus is recovered by gel electrophoresis. The fragments are ligated at 15°C, digested with *EcoRI* to eliminate *EcoRI* concatemers and cloned into the *EcoRI* site of dephosphorylated pCGN1761. The N/C-terminal deletion form of *NIM1* is under the transcriptional regulation of the double 35S promoter. Similarly, a chimeric form of *NIM1* is generated which consists of the S55/S59 mutagenized putative phosphorylation sites (Example 27) fused to the C-terminal deletion (Example 29). The construct is generated as described above. The constructs are sequenced to verify the fidelity of the start and stop codons and to confirm that no mutations occurred during cloning. The respective transformation cassettes including the 35S promoter, *NIM1* chimera and *tml* terminator are released from pCGN1761 by partial restriction digestion with XbaI and ligated into the XbaI site of dephosphorylated pCIB200. SEQ ID NO's:28 and 29 show the DNA coding sequence and encoded amino acid sequence, respectively, of an altered form of the *NIM1* gene having both N-terminal and C-terminal amino acid deletions.

The present invention also encompasses altered forms of alleles of *NIM1*, wherein the coding sequence of such an allele hybridizes under the following conditions to the coding sequence set forth in SEQ ID NO:28: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C. In these embodiments, alleles of *NIM1* hybridizing to SEQ ID NO:28 under the above conditions are altered so that the encoded product has both an N-terminal deletion, which removes lysine residues that may serve as potential ubiquitination sites in addition to the serines at amino acid positions corresponding to positions 55 and 59 of the wild-type gene product, as well as a C-terminal deletion, which removes serine and threonine residues.

Example 31: Generation Of Altered Forms Of *NIM1* - Ankyrin Domains

NIM1 exhibits homology to ankyrin motifs at approximately amino acids 103-362. Using the method of Ho *et al.* (1989), the DNA sequence encoding the putative ankyrin domains (SEQ ID NO:2: 3093-3951) is PCR amplified (conditions: 94°C 3 min:35x (94°C 30 sec: 62°C 30 sec: 72°C 2 min): 72°C 10 min) from the *NIM1* cDNA (SEQ ID NO:21: 349-1128) using the following primers: 5'-ggaattcaATGGACTCCAACAACACCGCCGC-3' (SEQ ID NO:38) and 5' ggaattcTCAACCTTCCAAAGTTGCTTCTGATG-3' (SEQ ID NO:39). The resulting product is restriction endonuclease digested with *EcoRI* and then spliced into the *EcoRI* site of dephosphorylated pCGN1761 under the transcriptional regulation of the double 35S promoter. The construct is sequenced to verify the presence of the synthetic start codon (ATG), an in-frame stop codon (TGA) and to confirm that no other mutations occurred during PCR. The transformation cassette including the 35S promoter, ankyrin domains, and *tml* terminator is released from pCGN1761 by partial restriction digestion with *XbaI* and ligated into the *XbaI* site of dephosphorylated pCIB200. SEQ ID NO's:30 and 31 show the DNA coding sequence and encoded amino acid sequence, respectively, of the ankyrin domain of *NIM1*.

The present invention also encompasses altered forms of alleles of *NIM1*, wherein the coding sequence of such an allele hybridizes under the following conditions to the coding sequence set forth in SEQ ID NO:30: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55°C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C. In these embodiments, alleles of *NIM1* hybridizing to SEQ ID NO:30 under the above conditions are altered so that the encoded product consists essentially of the ankyrin domains of the wild-type gene product.

Example 32: Construction Of Chimeric Genes

To increase the likelihood of appropriate spatial and temporal expression of altered *NIM1* forms, a 4407 bp HindIII/BamHI fragment (SEQ ID NO:2: bases 1249-5655) and/or a 5655 bp EcoRV/BamHI fragment (SEQ ID NO:2: bases 1-5655) containing the *NIM1* promoter and gene is used for the creation of the altered *NIM1* forms in Examples 27-31 above. Although the construction steps may differ, the concepts are comparable to the examples previously described herein. Strong overexpression of the altered forms may potentially be lethal. Therefore, the altered forms of the *NIM1* gene described in Examples 27-31 may be placed under the regulation of promoters other than the endogenous *NIM1* promoter, including but not limited to the *nos* promoter or small subunit of Rubisco promoter. Likewise, the altered *NIM1* forms may be expressed under the regulation of the pathogen-responsive promoter PR-1 (U.S. Pat. No. 5,614,395). Such expression permits strong expression of the altered *NIM1* forms only under pathogen attack or other SAR-activating conditions. Furthermore, disease resistance may be evident in the transformants expressing altered *NIM1* forms under PR-1 promoter regulation when treated with concentrations of SAR activator compounds (i.e., BTH or INA) which normally do not activate SAR, thereby activating a feedback loop (Weymann et al., (1995) Plant Cell 7: 2013-2022).

Example 33: Transformation Of Altered Forms Of The *NIM1* Into *Arabidopsis thaliana*

The constructs generated (Examples 27-32) are moved into *Agrobacterium tumefaciens* by electroporation into strain GV3101. These constructs are used to transform *Arabidopsis* ecotypes Col-0 and Ws-0 by vacuum infiltration (Mindrinos et al., *Cell* 78, 1089-1099 (1994)) or by standard root transformation. Seed from these plants is harvested and allowed to germinate on agar plates with kanamycin (or another appropriate antibiotic) as selection agent. Only plantlets that are transformed with cosmid DNA can detoxify the selection agent and survive. Seedlings that survive the selection are transferred to soil and tested for a CIM (constitutive immunity) phenotype. Plants are evaluated for observable phenotypic differences compared to wild type plants.

Example 34: Assessment Of CIM Phenotype In Plants Transformed With Altered Forms Of *NIM1*

A leaf from each primary transformant is harvested, RNA is isolated (Verwoerd et al., 1989, Nuc Acid Res, 2362) and tested for constitutive PR-1 expression by RNA blot analysis (Uknes et al., 1992). Each transformant is evaluated for an enhanced disease resistance response indicative of constitutive SAR expression analysis (Uknes et al., 1992). Conidial suspensions of 5-10x10^4 spores/ml from two compatible *P. parasitica* isolates, Emwa and Noco (i.e. these fungal strains cause disease on wildtype Ws-O and Col-0 plants, respectively), are prepared, and transformants are sprayed with the appropriate isolate depending on the ecotype of the transformant. Inoculated plants are incubated under high humidity for 7 days. Plants are disease rated at day 7 and a single leaf is harvested for RNA blot analysis utilizing a probe which provides a means to measure fungal infection.

Transformants that exhibit a CIM phenotype are taken to the T1 generation and homozygous plants are identified. Transformants are subjected to a battery of disease resistance tests as described below. Fungal infection with Noco and Emwa is repeated and leaves are stained with lactophenol blue to identify the presence of fungal hyphae as described in Dietrich et al., (1994). Transformants are infected with the bacterial pathogen *Pseudomonas syringae* DC3000 to evaluate the spectrum of resistance evident as described in Uknes et al. (1993). Uninfected plants are evaluated for both free and glucose-conjugated SA and leaves are stained with lactophenol blue to evaluate for the presence of microscopic lesions. Resistant plants are sexually crossed with SAR mutants such as NahG (U.S. Pat. No. 5,614,395) and *ndr1* to establish the epistatic relationship of the resistance phenotype to other mutants and evaluate how these dominant-negative mutants of *NIM1* may influence the SA-dependent feedback loop.

Example 35: Isolation Of *NIM1* Homologs

Using the *NIM1* cDNA (SEQ ID NO:21) as a probe, homologs of Arabidopsis *NIM1* are identified through screening genomic or cDNA libraries from different crops such as, but not limited to those listed below in Example 36. Standard techniques for accomplishing this include hybridization screening of plated DNA libraries (either plaques or colonies; see, *e.g.* Sambrook *et al.*, Molecular Cloning , eds., Cold Spring Harbor Laboratory Press. (1989)) and amplification by PCR using oligonucleotide primers (see, *e.g.* Innis *et al.*, PCR Protocols, a Guide to Methods and Applications eds., Academic Press (1990)). Homologs

identified are genetically engineered into the expression vectors herein and transformed into the above listed crops. Transformants are evaluated for enhanced disease resistance using relevant pathogens of the crop plant being tested.

NIM1 homologs in the genomes of cucumber, tomato, tobacco, maize, wheat and barley have been detected by DNA blot analysis. Genomic DNA was isolated from cucumber, tomato, tobacco, maize, wheat and barley, restriction digested with the enzymes BamHI, HindIII, XbaI, or SalI, electrophoretically separated on 0.8% agarose gels and transferred to nylon membrane by capillary blotting. Following UV-crosslinking to affix the DNA, the membrane was hybridized under low stringency conditions [(1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride) at 55°C for 18-24h] with ^{32}P-radiolabelled *Arabidopsis thaliana NIM1* cDNA. Following hybridization the blots were washed under low stringency conditions [6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55°C; 1XSSC is 0.15M NaCl, 15mM Na-citrate (pH7.0)] and exposed to X-ray film to visualize bands that correspond to *NIM1*.

In addition, expressed sequence tags (EST) identified with similarity to the *NIM1* gene can be used to isolate homologues. For example, several rice expressed sequence tags (ESTs) have been identified with similarity to the *NIM1* gene. A multiple sequence alignment was constructed using Clustal V (Higgins, Desmond G. and Paul M. Sharp (1989), Fast and sensitive multiple sequence alignments on a microcomputer, CABIOS 5:151-153) as part of the DNA* (1228 South Park Street, Madison Wisconsin, 53715) Lasergene Biocomputing Software package for the Macintosh (1994). Certain regions of the *NIM1* protein are homologous in amino acid sequence to 4 different rice cDNA protein products. The homologies were identified using the *NIM1* sequences in a GenBank BLAST search. Comparisons of the regions of homology in *NIM1* and the rice cDNA products are shown in Figure 8 (*See also*, SEQ ID NO:3 and SEQ ID NO's:4-11). The NIM1 protein fragments show from 36 to 48% identical amino acid sequences with the 4 rice products. These rice EST's may be especially useful for isolation of *NIM1* homologues from other monocots.

Homologues may be obtained by PCR. In this method, comparisons are made between known homologues (e.g., rice and Arabidopsis). Regions of high amino acid and DNA similarity or identity are then used to make PCR primers. Regions rich in amino acid residues M and W are best followed by regions rich in amino acid residues F, Y, C, H, Q, K and E because these amino acids are encoded by a limited number of codons. Once a suitable region is identified, primers for that region are made with a diversity of substitutions in the 3rd codon position. This diversity of substitution in the third position may be

constrained depending on the species that is being targeted. For example, because maize is GC rich, primers are designed that utilize a G or a C in the 3rd position, if possible.

The PCR reaction is performed from cDNA or genomic DNA under a variety of standard conditions. When a band is apparent, it is cloned and/or sequenced to determine if it is a *NIM1* homologue.

Example 36: Expression Altered Forms Of *NIM1* In Crop Plants

Those constructs conferring a CIM phenotype in Col-0 or Ws-0 are transformed into crop plants for evaluation. Alternatively, altered native *NIMI* genes isolated from crops in the preceding example are put back into the respective crops. Although the *NIM1* gene can be inserted into any plant cell falling within these broad classes, it is particularly useful in crop plant cells, such as rice, wheat, barley, rye, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane. Transformants are evaluated for enhanced disease resistance. In a preferred embodiment of the invention, the expression of the altered form of the *NIM1* gene is at a level which is at least two-fold above the expression level of the native *NIM1* gene in wild type plants and is preferably ten-fold above the wild type expression level.

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The disclosures of each of the following references are hereby expressly incorporated by reference into the instant disclosure:

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Novartis AG
(B) STREET: Schwarzwaldallee 215
(C) CITY: Basel
(E) COUNTRY: Switzerland
(F) POSTAL CODE (ZIP): 4002
(G) TELEPHONE: +41 61 69 11 11
(H) TELEFAX: + 41 61 696 79 76
(I) TELEX: 962 991

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(ii) TITLE OF INVENTION: METHODS OF USING THE NIM1 GENE TO CONFER DISEASE RESISTANCE IN PLANTS

(iii) NUMBER OF SEQUENCES: 39

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9919 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CGACTTTTCA AAACTGCTCT TTAAAACCCA TAATCTGTGA ACATCTTCTT GATTGTTGTT 8220

TATCCAGTGA CGAATAACAC CTAGCTTCCC TTCGTAGCTG ACTAACTCTG GGAATAAACC 8280

AACGTTTGGA GTATGTAAGA AAGACCAAGT TTCGGTTTTG GGACATAACC GGATCACATT 8340

GTGGTTCCAT GATCTCCAAT GCAAGAACCC TGAAGCTTGT ACCGGGTTTG AAAGAATTAG 8400

ACCGTCTGTT CTCGGTAGAC GCAAATTTTT TAATCTCTTC CACATAAACG AATCGGAATC 8460

AAAAACTTCG CACGCAAAAG TTCTGAGATT CCGAGTCATA CCAGGCGATT TCGAAAGCCT 8520

AAATATTTTA TACCGGAAAG GCTGCAATCC GGTTACCGTT AGACCTAATG ACTTATCACA 8580

ACTCCTCACT TTTGGGTTTG GTATGATCTG ATACTGTTTT GTTGTTGGTT TGCAGACTAT 8640

GTATTCCGGT ATTGGTCTTG TATCATTATA ACAAAGCAAT ATCCCATGAC GTGCATCACA 8700

AGCTTTGATC TTTACCTCTC CTTGTGGCAG AAAATCGATG GAGACTCCTT TGTTATCCAA 8760

ATCTCTCCTC TCATGGAAAA AACTGGTATC AAGTTTGTAT CCTCTTTCGT AGCGTTCTAG 8820

GAAGTATCCA GAGATATTGT TGGTTCGATG GAGATTTAGG TTGACAAACC AAGACTCGTA 8880

GCTTCTCTTG TTGCACTCTT TATTGATGAG CCTCAATTTT CCGATTTCGG ACCCCCGAAG 8940

ATAAGAAAGA ACCTCTTGGA TCGTGTCCTG ATTTATCACC GGAGAACTCA TGATCTTATT 9000

GGAAAAAAGA AAGAAAGAGA TGAGCACGAT CAGTGAATGA GATATATAGA AATCAGGATT 9060

GGTAGAGAAC CGACGATGAT GAATATACAA GTGTTTATAA GTATCACAAA TTGCCTTTTT 9120

CTTCGCTAGT CCCAAAACAA GCAAATTAAC CAAAGATAAA ATCTTCATTA ATGTTTTCCT 9180

TTTTCTTCGC CAGTCCCAGA TAAAAATATA TATAAAATAT TTCATTAGGT TACTTGTAGT 9240

ACCTTGAGCC CAAAGTTTCT CTTTTGACTT TTAACCAAAT TAACAGTAAT TTAATAGCTA 9300

GACTTAGAAA ACAACATTTT GTATATATAT TCTTTGACAT CAAAATTCAA CAATCTTTGG 9360

GTTTCTATAG TGTTTTTTTT CTTATTCTAA TAGATTACCA CTCATTATAT CATATACAAA 9420

GTGTTTCCTT TTCAATCAAC ATCCATTTTC TTTAAAAATT AGCAAGTTTG TTCTTATATC 9480

ATCATTCAGC AGATTTCTTA ATTAAACTTA GTGATTTCCA TTTTGCACCT ATATGTTTCT 9540

CTTTCTTAGT TTAGTACTTT AAATTTTCAT ATATATAATT TATTAAAATT AAAAGTAAAA 9600

ACTCCAGTTT AACTTATGTT AAATGTTTCA TCACACTAAA AGAGCATTAA GTAATAAATA 9660

TTTTAGCTTT ATGAAAAAAA ATATCAAATC ACTGAAGACA TTTGTTGGCC TATACTCTAT 9720

TTTTTATTTG GCCAATTAGT AATAGACTAA TAGTAACTCA TATGATATCT CTCTAATTCT 9780

GGCGAAACGA ATATTCTGAT TCTAAAGATA GTAAAAATGA ATTTTGATGA AGGGAATACT 9840

ATTTCACACA CCTAGAAAGA GTAAGGTAGA AACCTTTTTT TTTTTGGTCA GATTCTTGTA 9900

TCAAGAAGTT CTCATCGAT 9919

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5655 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 2787..3347
(D) OTHER INFORMATION: /product= "1st exon of NIM1"

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 3427..4162
(D) OTHER INFORMATION: /product= "2nd exon of NIM1"

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 4271..4474
(D) OTHER INFORMATION: /product= "3rd exon of NIM1"

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 4586..4866
(D) OTHER INFORMATION: /product= "4th exon of NIM1"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join(2787..3347, 3427..4162, 4271..4474, 4586..4866)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGTGATGCAA GTCATGGGAT ATTGCTTTGT GTTAAGTATA CAAAACCATC ACGTGGATAC 60

ATAGTCTTCA AACCAACCAC TAAACAGTAT CAGGTCATAC CAAAGCCAGA AGTGAAGGGT 120

TGGGATATGT CATTGGGTTT AGCGGTAATC GGATTGAACC CTTTCCGGTA TAAAATACAA 180

AGGCTTTCGC AGTCTCGGCG TATGTGTATG TCTCGGGGTA TCTACCATTT GAATCACAGA 240

ACTTTTATGT GCGAAGTTTT CGATTCTGAT TCGTTTACCT GGAAGAGATT AGAAAATTTG 300

CGTCTACCAA AAACAGACAG ATTAATTTTT TCCAACCCGA TACAAGTTTC GGGGTTCTTG 360

CATTGGATAT CACGGAACAA CAATGTGATC CGGTTTTGTC TCAAAACCGA AACTTGGTCC 420

TTCTTCCATA CTCCGAACTC TGATGTTTTC TCAGGATTAG TCAGATACGA AGGGAAGCTA 480

GGTGCTATTC GTCAGTGGAC AAACAAAGAT CAAGAAGATG TTCACGAGTT ATGGGTTTTA 540

AAGAGCAGTT TTGAAAAGTC GTGGGTTAAA GTGAAAGATA TTAAAAGCAT TGGAGTAGAT 600

TTGATTACGT GGACTCCAAG CAACGACGTT GTATTGTTTC GTAGTAGTGA TCGTGGTTGC 660

CTCTACAACA TAAACGCAGA GAAGTTGAAT TTAGTTTATG CAAAAAAAGA GGGATCTGAT 720

TGTTCTTTCG TTTGTTTTCC GTTTTGTTCT GATTACGAGA GGGTTGATCT GAACGGAAGA 780

AGCAACGGGC CGACACTTTA AAAAAAAAAT AAAAAAAATG GGCCGACAAA TGCAAACGTA 840

GTTGACAAGG ATCTCAAGTC TCAAGTCTCA ATTGGCTCGC TCATTGTGGG GCATAAATAT 900

ATCTAGTGAT GTTTAATTGT TTTTTATAAG GTAAAAAGGA ATATTGAATT TTGTTTCTTA 960

GGTTTATGTA ATAATACCAA ACATTGTTTT ATGAATATTT AATCTGATTT TTTGGCTAGT 1020

TATTTTATTA TATCAAGGGT TCCTGTTTAT AGTTGAAAAC AGTTACTGTA TAGAAAATAG 1080

TGTCCCAATT TTCTCTCTTA AATAATATAT TAGTTAATAA AAGATATTTT AATATATTAG 1140

ATATACATAA TATCTAAAGC AACACATATT TAGACACAAC ACGTAATATC TTACTATTGT 1200

TTACATATAT TTATAGCTTA CCAATATAAC CCGTATCTAT GTTTTATAAG CTTTTATACA 1260

ATATATGTAC GGTATGCTGT CCACGTATAT ATATTCTCCA AAAAAAACGC ATGGTACACA 1320

AAATTTATTA AATATTTGGC AATTGGGTGT TTATCTAAAG TTTATCACAA TATTTATCAA 1380

CTATAATAGA TGGTAGAAGA TAAAAAAATT ATATCAGATT GATTCAATTA AATTTTATAA 1440

TATATCATTT TAAAAAATTA ATTAAAAGAA AACTATTTCA TAAAATTGTT CAAAAGATAA 1500

TTAGTAAAAT TAATTAAATA TGTGATGCTA TTGAGTTATA GAGAGTTATT GTAAATTTAC 1560

TTAAAATCAT ACAAATCTTA TCCTAATTTA ACTTATCATT TAAGAAATAC AAAAGTAAAA 1620

AACGCGGAAA GCAATAATTT ATTTACCTTA TTATAACTCC TATATAAAGT ACTCTGTTTA 1680

TTCAACATAA TCTTACGTTG TTGTATTCAT AGGCATCTTT AACCTATCTT TTCATTTTCT 1740

GATCTCGATC GTTTTCGATC CAACAAAATG AGTCTACCGG TGAGGAACCA AGAGGTGATT 1800

ATGCAGATTC CTTCTTCTTC TCAGTTTCCA GCAACATCGA GTCCGGAAAA CACCAATCAA 1860

GTGAAGGATG AGCCAAATTT GTTTAGACGT GTTATGAATT TGCTTTTACG TCGTAGTTAT 1920

TGAAAAAGCT GATTTATCGC ATGATTCAGA ACGAGAAGTT GAAGGCAAAT AACTAAAGAA 1980

GTCTTTTATA TGTATACAAT AATTGTTTTT AAATCAAATC CTAATTAAAA AAATATATTC 2040

ATTATGACTT TCATGTTTTT AATGTAATTT ATTCCTATAT CTATAATGAT TTTGTTGTGA 2100

AGAGCGTTTT CATTTGCTAT AGAACAAGGA GAATAGTTCC AGGAAATATT CGACTTGATT 2160

TAATTATAGT GTAAACATGC TGAACACTGA AAATTACTTT TTCAATAAAC GAAAAATATA 2220

ATATACATTA CAAAACTTAT GTGAATAAAG CATGAAACTT AATATACGTT CCCTTTATCA 2280

TTTTACTTCA AAGAAAATAA ACAGAAATGT AACTTTCACA TGTAAATCTA ATTCTTAAAT 2340

TTAAAAAATA ATATTTATAT ATTTATATGA AAATAACGAA CCGGATGAAA AATAAATTTT 2400

ATATATTTAT ATCATCTCCA AATCTAGTTT GGTTCAGGGG CTTACCGAAC GGGATTGAAC 2460

TTCTCATATA CAAAAATTAG CAACACAAAA TGTCTCCGGT ATAAATACTA ACATTTATAA 2520

CCCGAACCGG TTTAGCTTCC TGTTATATCT TTTTAAAAAA GATCTCTGAC AAAGATTCCT 2580

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TTCCTGGAAA TTTACCGGTT TTGGTGAAAT GTAAACCGTG GGACGAGGAT GCTTCTTCAT    2640

ATCTCACCAC CACTCTCGTT GACTTGACTT GGCTCTGCTC GTCAATGGTT ATCTTCGATC    2700

TTTAACCAAA TCCAGTTGAT AAGGTCTCTT CGTTGATTAG CAGAGATCTC TTTAATTTGT    2760

GAATTTCAAT TCATCGGAAC CTGTTG ATG GAC ACC ACC ATT GAT GGA TTC GCC      2813
                             Met Asp Thr Thr Ile Asp Gly Phe Ala
                               1               5

GAT TCT TAT GAA ATC AGC AGC ACT AGT TTC GTC GCT ACC GAT AAC ACC       2861
Asp Ser Tyr Glu Ile Ser Ser Thr Ser Phe Val Ala Thr Asp Asn Thr
 10                  15                  20                  25

GAC TCC TCT ATT GTT TAT CTG GCC GCC GAA CAA GTA CTC ACC GGA CCT      2909
Asp Ser Ser Ile Val Tyr Leu Ala Ala Glu Gln Val Leu Thr Gly Pro
                 30                  35                  40

GAT GTA TCT GCT CTG CAA TTG CTC TCC AAC AGC TTC GAA TCC GTC TTT      2957
Asp Val Ser Ala Leu Gln Leu Leu Ser Asn Ser Phe Glu Ser Val Phe
             45                  50                  55

GAC TCG CCG GAT GAT TTC TAC AGC GAC GCT AAG CTT GTT CTC TCC GAC      3005
Asp Ser Pro Asp Asp Phe Tyr Ser Asp Ala Lys Leu Val Leu Ser Asp
         60                  65                  70

GGC CGG GAA GTT TCT TTC CAC CGG TGC GTT TTG TCA GCG AGA AGC TCT      3053
Gly Arg Glu Val Ser Phe His Arg Cys Val Leu Ser Ala Arg Ser Ser
     75                  80                  85

TTC TTC AAG AGC GCT TTA GCC GCC GCT AAG AAG GAG AAA GAC TCC AAC      3101
Phe Phe Lys Ser Ala Leu Ala Ala Ala Lys Lys Glu Lys Asp Ser Asn
 90                  95                  100                 105

AAC ACC GCC GCC GTG AAG CTC GAG CTT AAG GAG ATT GCC AAG GAT TAC      3149
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Asn Thr Ala Ala Val Lys Leu Glu Leu Lys Glu Ile Ala Lys Asp Tyr
                110                 115                 120

GAA GTC GGT TTC GAT TCG GTT GTG ACT GTT TTG GCT TAT GTT TAC AGC      3197
Glu Val Gly Phe Asp Ser Val Val Thr Val Leu Ala Tyr Val Tyr Ser
            125                 130                 135

AGC AGA GTG AGA CCG CCG CCT AAA GGA GTT TCT GAA TGC GCA GAC GAG      3245
Ser Arg Val Arg Pro Pro Pro Lys Gly Val Ser Glu Cys Ala Asp Glu
        140                 145                 150

AAT TGC TGC CAC GTG GCT TGC CGG CCG GCG GTG GAT TTC ATG TTG GAG      3293
Asn Cys Cys His Val Ala Cys Arg Pro Ala Val Asp Phe Met Leu Glu
    155                 160                 165

GTT CTC TAT TTG GCT TTC ATC TTC AAG ATC CCT GAA TTA ATT ACT CTC      3341
Val Leu Tyr Leu Ala Phe Ile Phe Lys Ile Pro Glu Leu Ile Thr Leu
170                 175                 180                 185

TAT CAG GTAAAACACC ATCTGCATTA AGCTATGGTT ACACATTCAT GAATATGTTC      3397
Tyr Gln

TTACTTGAGT ACTTGTATTT GTATTTCAG AGG CAC TTA TTG GAC GTT GTA GAC      3450
                               Arg His Leu Leu Asp Val Val Asp
                                   190                 195

AAA GTT GTT ATA GAG GAC ACA TTG GTT ATA CTC AAG CTT GCT AAT ATA      3498
Lys Val Val Ile Glu Asp Thr Leu Val Ile Leu Lys Leu Ala Asn Ile
            200                 205                 210

TGT GGT AAA GCT TGT ATG AAG CTA TTG GAT AGA TGT AAA GAG ATT ATT      3546
Cys Gly Lys Ala Cys Met Lys Leu Leu Asp Arg Cys Lys Glu Ile Ile
        215                 220                 225

GTC AAG TCT AAT GTA GAT ATG GTT AGT CTT GAA AAG TCA TTG CCG GAA      3594
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Val Lys Ser Asn Val Asp Met Val Ser Leu Glu Lys Ser Leu Pro Glu
        230                 235                 240

GAG CTT GTT AAA GAG ATA ATT GAT AGA CGT AAA GAG CTT GGT TTG GAG      3642
Glu Leu Val Lys Glu Ile Ile Asp Arg Arg Lys Glu Leu Gly Leu Glu
    245                 250                 255

GTA CCT AAA GTA AAG AAA CAT GTC TCG AAT GTA CAT AAG GCA CTT GAC      3690
Val Pro Lys Val Lys Lys His Val Ser Asn Val His Lys Ala Leu Asp
260                 265                 270                 275

TCG GAT GAT ATT GAG TTA GTC AAG TTG CTT TTG AAA GAG GAT CAC ACC      3738
Ser Asp Asp Ile Glu Leu Val Lys Leu Leu Leu Lys Glu Asp His Thr
                280                 285                 290

AAT CTA GAT GAT GCG TGT GCT CTT CAT TTC GCT GTT GCA TAT TGC AAT      3786
Asn Leu Asp Asp Ala Cys Ala Leu His Phe Ala Val Ala Tyr Cys Asn
            295                 300                 305

GTG AAG ACC GCA ACA GAT CTT TTA AAA CTT GAT CTT GCC GAT GTC AAC      3834
Val Lys Thr Ala Thr Asp Leu Leu Lys Leu Asp Leu Ala Asp Val Asn
        310                 315                 320

CAT AGG AAT CCG AGG GGA TAT ACG GTG CTT CAT GTT GCT GCG ATG CGG      3882
His Arg Asn Pro Arg Gly Tyr Thr Val Leu His Val Ala Ala Met Arg
    325                 330                 335

AAG GAG CCA CAA TTG ATA CTA TCT CTA TTG GAA AAA GGT GCA AGT GCA      3930
Lys Glu Pro Gln Leu Ile Leu Ser Leu Leu Glu Lys Gly Ala Ser Ala
340                 345                 350                 355

TCA GAA GCA ACT TTG GAA GGT AGA ACC GCA CTC ATG ATC GCA AAA CAA      3978
Ser Glu Ala Thr Leu Glu Gly Arg Thr Ala Leu Met Ile Ala Lys Gln
                360                 365                 370

GCC ACT ATG GCG GTT GAA TGT AAT AAT ATC CCG GAG CAA TGC AAG CAT      4026
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Ala Thr Met Ala Val Glu Cys Asn Asn Ile Pro Glu Gln Cys Lys His
            375                 380                 385

TCT CTC AAA GGC CGA CTA TGT GTA GAA ATA CTA GAG CAA GAA GAC AAA      4074
Ser Leu Lys Gly Arg Leu Cys Val Glu Ile Leu Glu Gln Glu Asp Lys
        390                 395                 400

CGA GAA CAA ATT CCT AGA GAT GTT CCT CCC TCT TTT GCA GTG GCG GCC      4122
Arg Glu Gln Ile Pro Arg Asp Val Pro Pro Ser Phe Ala Val Ala Ala
    405                 410                 415

GAT GAA TTG AAG ATG ACG CTG CTC GAT CTT GAA AAT AGA G                4162
Asp Glu Leu Lys Met Thr Leu Leu Asp Leu Glu Asn Arg
420                 425                 430

GTATCTATCA AGTCTTATTT CTTATATGTT TGAATTAAAT TTATGTCCTC TCTATTAGGA    4222

AACTGAGTGA ACTAATGATA ACTATTCTTT GTGTCGTCCA CTGTTTAG  TT GCA CTT     4278
                                                    Val Ala Leu
                                                            435

GCT CAA CGT CTT TTT CCA ACG GAA GCA CAA GCT GCA ATG GAG ATC GCC      4326
Ala Gln Arg Leu Phe Pro Thr Glu Ala Gln Ala Ala Met Glu Ile Ala
                440                 445                 450

GAA ATG AAG GGA ACA TGT GAG TTC ATA GTG ACT AGC CTC GAG CCT GAC      4374
Glu Met Lys Gly Thr Cys Glu Phe Ile Val Thr Ser Leu Glu Pro Asp
            455                 460                 465

CGT CTC ACT GGT ACG AAG AGA ACA TCA CCG GGT GTA AAG ATA GCA CCT      4422
Arg Leu Thr Gly Thr Lys Arg Thr Ser Pro Gly Val Lys Ile Ala Pro
        470                 475                 480

TTC AGA ATC CTA GAA GAG CAT CAA AGT AGA CTA AAA GCG CTT TCT AAA      4470
Phe Arg Ile Leu Glu Glu His Gln Ser Arg Leu Lys Ala Leu Ser Lys
    485                 490                 495
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ACC G GTATGGATTC TCACCCACTT CATCGGACTC CTTATCACAA AAAACAAAAC          4524
Thr
500

TAAATGATCT TTAAACATGG TTTTGTTACT TGCTGTCTGA CCTTGTTTTT TTTATCATCA    4584

G  TG GAA CTC GGG AAA CGA TTC TTC CCG CGC TGT TCG GCA GTG CTC         4629
  Val Glu Leu Gly Lys Arg Phe Phe Pro Arg Cys Ser Ala Val Leu
                  505                 510                 515

GAC CAG ATT ATG AAC TGT GAG GAC TTG ACT CAA CTG GCT TGC GGA GAA      4677
Asp Gln Ile Met Asn Cys Glu Asp Leu Thr Gln Leu Ala Cys Gly Glu
                520                 525                 530

GAC GAC ACT GCT GAG AAA CGA CTA CAA AAG AAG CAA AGG TAC ATG GAA      4725
Asp Asp Thr Ala Glu Lys Arg Leu Gln Lys Lys Gln Arg Tyr Met Glu
            535                 540                 545

ATA CAA GAG ACA CTA AAG AAG GCC TTT AGT GAG GAC AAT TTG GAA TTA      4773
Ile Gln Glu Thr Leu Lys Lys Ala Phe Ser Glu Asp Asn Leu Glu Leu
        550                 555                 560

GGA AAT TCG TCC CTG ACA GAT TCG ACT TCT TCC ACA TCG AAA TCA ACC      4821
Gly Asn Ser Ser Leu Thr Asp Ser Thr Ser Ser Thr Ser Lys Ser Thr
    565                 570                 575

GGT GGA AAG AGG TCT AAC CGT AAA CTC TCT CAT CGT CGT CGG TGA          4866
Gly Gly Lys Arg Ser Asn Arg Lys Leu Ser His Arg Arg Arg  *
580                 585                 590

GACTCTTGCC TCTTAGTGTA ATTTTTGCTG TACCATATAA TTCTGTTTTC ATGATGACTG    4926

TAACTGTTTA TGTCTATCGT TGGCGTCATA TAGTTTCGCT CTTCGTTTTG CATCCTGTGT    4986

ATTATTGCTG CAGGTGTGCT TCAAACAAAT GTTGTAACAA TTTGAACCAA TGGTATACAG    5046
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ATTTGTAATA TATATTTATG TACATCAACA ATAACCCATG ATGGTGTTAC AGAGTTGCTA 5106

GAATCAAAGT GTGAAATAAT GTCAAATTGT TCATCTGTTG GATATTTTCC ACCAAGAACC 5166

AAAAGAATAT TCAAGTTCCC TGAACTTCTG GCAACATTCA TGTTATATGT ATCTTCCTAA 5226

TTCTTCCTTT AACCTTTTGT AACTCGAATT ACACAGCAAG TTAGTTTCAG GTCTAGAGAT 5286

AAGAGAACAC TGAGTGGGCG TGTAAGGTGC ATTCTCCTAG TCAGCTCCAT TGCATCCAAC 5346

ATTTGTGAAT GACACAAGTT AACAATCCTT TGCACCATTT CTGGGTGCAT ACATGGAAAC 5406

TTCTTCGATT GAAACTTCCC ACATGTGCAG GTGCGTTCGC TGTCACTGAT AGACCAAGAG 5466

ACTGAAAGCT TTCACAAATT GCCCTCAAAT CTTCTGTTTC TATCGTCATG ACTCCATATC 5526

TCCGACCACT GGTCATGAGC CAGAGCCCAC TGATTTTGAG GGAATTGGGC TAACCATTTC 5586

CGAGCTTCTG AGTCCTTCTT TTTGATGTCC TTTATGTAGG AATCAAATTC TTCCTTCTGA 5646

CTTGTGGAT 5655

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 594 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Asp Thr Thr Ile Asp Gly Phe Ala Asp Ser Tyr Glu Ile Ser Ser

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1               5                  10                  15

Thr Ser Phe Val Ala Thr Asp Asn Thr Asp Ser Ser Ile Val Tyr Leu
            20                  25                  30

Ala Ala Glu Gln Val Leu Thr Gly Pro Asp Val Ser Ala Leu Gln Leu
        35                  40                  45

Leu Ser Asn Ser Phe Glu Ser Val Phe Asp Ser Pro Asp Asp Phe Tyr
    50                  55                  60

Ser Asp Ala Lys Leu Val Leu Ser Asp Gly Arg Glu Val Ser Phe His
65                  70                  75                  80

Arg Cys Val Leu Ser Ala Arg Ser Ser Phe Phe Lys Ser Ala Leu Ala
                85                  90                  95

Ala Ala Lys Lys Glu Lys Asp Ser Asn Asn Thr Ala Ala Val Lys Leu
            100                 105                 110

Glu Leu Lys Glu Ile Ala Lys Asp Tyr Glu Val Gly Phe Asp Ser Val
        115                 120                 125

Val Thr Val Leu Ala Tyr Val Tyr Ser Ser Arg Val Arg Pro Pro Pro
    130                 135                 140

Lys Gly Val Ser Glu Cys Ala Asp Glu Asn Cys Cys His Val Ala Cys
145                 150                 155                 160

Arg Pro Ala Val Asp Phe Met Leu Glu Val Leu Tyr Leu Ala Phe Ile
                165                 170                 175

Phe Lys Ile Pro Glu Leu Ile Thr Leu Tyr Gln Arg His Leu Leu Asp
            180                 185                 190

Val Val Asp Lys Val Val Ile Glu Asp Thr Leu Val Ile Leu Lys Leu
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195 200 205

Ala Asn Ile Cys Gly Lys Ala Cys Met Lys Leu Leu Asp Arg Cys Lys
210 215 220

Glu Ile Ile Val Lys Ser Asn Val Asp Met Val Ser Leu Glu Lys Ser
225 230 235 240

Leu Pro Glu Glu Leu Val Lys Glu Ile Ile Asp Arg Arg Lys Glu Leu
245 250 255

Gly Leu Glu Val Pro Lys Val Lys Lys His Val Ser Asn Val His Lys
260 265 270

Ala Leu Asp Ser Asp Asp Ile Glu Leu Val Lys Leu Leu Leu Lys Glu
275 280 285

Asp His Thr Asn Leu Asp Asp Ala Cys Ala Leu His Phe Ala Val Ala
290 295 300

Tyr Cys Asn Val Lys Thr Ala Thr Asp Leu Leu Lys Leu Asp Leu Ala
305 310 315 320

Asp Val Asn His Arg Asn Pro Arg Gly Tyr Thr Val Leu His Val Ala
325 330 335

Ala Met Arg Lys Glu Pro Gln Leu Ile Leu Ser Leu Leu Glu Lys Gly
340 345 350

Ala Ser Ala Ser Glu Ala Thr Leu Glu Gly Arg Thr Ala Leu Met Ile
355 360 365

Ala Lys Gln Ala Thr Met Ala Val Glu Cys Asn Asn Ile Pro Glu Gln
370 375 380

Cys Lys His Ser Leu Lys Gly Arg Leu Cys Val Glu Ile Leu Glu Gln

385 390 395 400

Glu Asp Lys Arg Glu Gln Ile Pro Arg Asp Val Pro Pro Ser Phe Ala
405 410 415

Val Ala Ala Asp Glu Leu Lys Met Thr Leu Leu Asp Leu Glu Asn Arg
420 425 430

Val Ala Leu Ala Gln Arg Leu Phe Pro Thr Glu Ala Gln Ala Ala Met
435 440 445

Glu Ile Ala Glu Met Lys Gly Thr Cys Glu Phe Ile Val Thr Ser Leu
450 455 460

Glu Pro Asp Arg Leu Thr Gly Thr Lys Arg Thr Ser Pro Gly Val Lys
465 470 475 480

Ile Ala Pro Phe Arg Ile Leu Glu Glu His Gln Ser Arg Leu Lys Ala
485 490 495

Leu Ser Lys Thr Val Glu Leu Gly Lys Arg Phe Phe Pro Arg Cys Ser
500 505 510

Ala Val Leu Asp Gln Ile Met Asn Cys Glu Asp Leu Thr Gln Leu Ala
515 520 525

Cys Gly Glu Asp Asp Thr Ala Glu Lys Arg Leu Gln Lys Lys Gln Arg
530 535 540

Tyr Met Glu Ile Gln Glu Thr Leu Lys Lys Ala Phe Ser Glu Asp Asn
545 550 555 560

Leu Glu Leu Gly Asn Ser Ser Leu Thr Asp Ser Thr Ser Ser Thr Ser
565 570 575

Lys Ser Thr Gly Gly Lys Arg Ser Asn Arg Lys Leu Ser His Arg Arg

580 585 590

Arg *

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ile Arg Arg Met Arg Arg Ala Leu Asp Ala Ala Asp Ile Glu Leu Val
1 5 10 15

Lys Leu Met Val Met Gly Glu Gly Leu Asp Leu Asp Asp Ala Leu Ala
20 25 30

Val His Tyr Ala Val Gln His Cys Asn
35 40

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Pro Thr Gly Lys Thr Ala Leu His Leu Ala Ala Glu Met Val Ser Pro
1 5 10 15

Asp Met Val Ser Val Leu Leu Asp His His Ala Asp Xaa Asn Phe Arg
20 25 30

Thr Xaa Asp Gly Val Thr
35

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ile Arg Arg Met Arg Arg Ala Leu Asp Ala Ala Asp Ile Glu Leu Val
1 5 10 15

Lys Leu Met Val Met Gly Glu Gly Leu Asp Leu Asp Asp Ala Leu Ala

20 25 30

Val His Tyr Ala Val Gln His Cys Asn
35 40

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Arg Arg Pro Asp Ser Lys Thr Ala Leu His Leu Ala Ala Glu Met Val
1 5 10 15

Ser Pro Asp Met Val Ser Val Leu Leu Asp Gln
20 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ile Arg Arg Met Arg Arg Ala Leu Asp Ala Ala Asp Ile Glu Leu Val
1 5 10 15

Lys Leu Met Val Met Gly Glu Gly Leu Asp Leu Asp Asp Ala Leu Ala
20 25 30

Val His Tyr Ala Val Gln His Cys Asn
35 40

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Arg Arg Pro Asp Ser Lys Thr Ala Leu His Leu Ala Ala Glu Met Val
1 5 10 15

Ser Pro Asp Met Val Ser Val Leu Leu Asp Gln
20 25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ile Arg Arg Met Arg Arg Ala Leu Asp Ala Ala Asp Ile Glu Leu Val
1 5 10 15

Lys Leu Met Val Met Gly Glu Gly Leu Asp Leu Asp Asp Ala Leu Ala
20 25 30

Val His Tyr Ala Val Gln His Cys Asn
35 40

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Pro Thr Gly Lys Thr Ala Leu His Leu Ala Ala Glu Met Val Ser Pro
1 5 10 15

Asp Met Val

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AATTCTAAAG CATGCCGATC GG 22

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AATTCCGATC GGCATGCTTT A 21

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AATTCTAAAC CATGGCGATC GG 22

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AATTCCGATC GCCATGGTTT A 21

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCAGCTGGAA TTCCG 15

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CGGAATTCCA GCTGGCATG 19

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 314 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Phe Gln Pro Ala Gly His Gly Gln Asp Trp Ala Met Glu Gly Pro
1 5 10 15

Arg Asp Gly Leu Lys Lys Glu Arg Leu Val Asp Asp Arg His Asp Ser
20 25 30

Gly Leu Asp Ser Met Lys Asp Glu Glu Tyr Glu Gln Met Val Lys Glu
35 40 45

Leu Arg Glu Ile Arg Leu Gln Pro Gln Glu Ala Pro Leu Ala Ala Glu
50 55 60

Pro Trp Lys Gln Gln Leu Thr Glu Asp Gly Asp Ser Phe Leu His Leu

65 70 75 80

Ala Ile Ile His Glu Glu Lys Pro Leu Thr Met Glu Val Ile Gly Gln
85 90 95

Val Lys Gly Asp Leu Ala Phe Leu Asn Phe Gln Asn Asn Leu Gln Gln
100 105 110

Thr Pro Leu His Leu Ala Val Ile Thr Asn Gln Pro Gly Ile Ala Glu
115 120 125

Ala Leu Leu Lys Ala Gly Cys Asp Pro Glu Leu Arg Asp Phe Arg Gly
130 135 140

Asn Thr Pro Leu His Leu Ala Cys Glu Gln Gly Cys Leu Ala Ser Val
145 150 155 160

Ala Val Leu Thr Gln Thr Cys Thr Pro Gln His Leu His Ser Val Leu
165 170 175

Gln Ala Thr Asn Tyr Asn Gly His Thr Cys Leu His Leu Ala Ser Thr
180 185 190

His Gly Tyr Leu Ala Ile Val Glu His Leu Val Thr Leu Gly Ala Asp
195 200 205

Val Asn Ala Gln Glu Pro Cys Asn Gly Arg Thr Ala Leu His Leu Ala
210 215 220

Val Asp Leu Gln Asn Pro Asp Leu Val Ser Leu Leu Leu Lys Cys Gly
225 230 235 240

Ala Asp Val Asn Arg Val Thr Tyr Gln Gly Tyr Ser Pro Tyr Gln Leu
245 250 255

Thr Trp Gly Arg Pro Ser Thr Arg Ile Gln Gln Gln Leu Gly Gln Leu

260 265 270

Thr Leu Glu Asn Leu Gln Met Leu Pro Glu Ser Glu Asp Glu Glu Ser
275 280 285

Tyr Asp Thr Glu Ser Glu Phe Thr Glu Asp Glu Leu Pro Tyr Asp Asp
290 295 300

Cys Val Phe Gly Gly Gln Arg Leu Thr Leu
305 310

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 314 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Met Phe Gln Pro Ala Gly His Gly Gln Asp Trp Ala Met Glu Gly Pro
1 5 10 15

Arg Asp Gly Leu Lys Lys Glu Arg Leu Val Asp Asp Arg His Asp Ser
20 25 30

Gly Leu Asp Ser Met Lys Asp Glu Asp Tyr Glu Gln Met Val Lys Glu
35 40 45

Leu Arg Glu Ile Arg Leu Gln Pro Gln Glu Ala Pro Leu Ala Ala Glu
50 55 60

Pro Trp Lys Gln Gln Leu Thr Glu Asp Gly Asp Ser Phe Leu His Leu
65 70 75 80

Ala Ile Ile His Glu Glu Lys Thr Leu Thr Met Glu Val Ile Gly Gln
85 90 95

Val Lys Gly Asp Leu Ala Phe Leu Asn Phe Gln Asn Asn Leu Gln Gln
100 105 110

Thr Pro Leu His Leu Ala Val Ile Thr Asn Gln Pro Gly Ile Ala Glu
115 120 125

Ala Leu Leu Lys Ala Gly Cys Asp Pro Glu Leu Arg Asp Phe Arg Gly
130 135 140

Asn Thr Pro Leu His Leu Ala Cys Glu Gln Gly Cys Leu Ala Ser Val
145 150 155 160

Ala Val Leu Thr Gln Thr Cys Thr Pro Gln His Leu His Ser Val Leu
165 170 175

Gln Ala Thr Asn Tyr Asn Gly His Thr Cys Leu His Leu Ala Ser Ile
180 185 190

His Gly Tyr Leu Gly Ile Val Glu His Leu Val Thr Leu Gly Ala Asp
195 200 205

Val Asn Ala Gln Glu Pro Cys Asn Gly Arg Thr Ala Leu His Leu Ala
210 215 220

Val Asp Leu Gln Asn Pro Asp Leu Val Ser Leu Leu Leu Lys Cys Gly
225 230 235 240

Ala Asp Val Asn Arg Val Thr Tyr Gln Gly Tyr Ser Pro Tyr Gln Leu
245 250 255

Thr Trp Gly Arg Pro Ser Thr Arg Ile Gln Gln Gln Leu Gly Gln Leu
260 265 270

Thr Leu Glu Asn Leu Gln Thr Leu Pro Glu Ser Glu Asp Glu Glu Ser
275 280 285

Tyr Asp Thr Glu Ser Glu Phe Thr Glu Asp Glu Leu Pro Tyr Asp Asp
290 295 300

Cys Val Phe Gly Gly Gln Arg Leu Thr Leu
305 310

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 314 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Phe Gln Pro Ala Glu Pro Gly Gln Glu Trp Ala Met Glu Gly Pro
1 5 10 15

Arg Asp Ala Leu Lys Lys Glu Arg Leu Leu Asp Asp Arg His Asp Ser

20 25 30

Gly Leu Asp Ser Met Lys Asp Glu Glu Tyr Glu Gln Met Val Lys Glu
35 40 45

Leu Arg Glu Ile Arg Leu Glu Pro Gln Glu Ala Pro Arg Gly Ala Glu
50 55 60

Pro Trp Lys Gln Gln Leu Thr Glu Asp Gly Asp Ser Phe Leu His Leu
65 70 75 80

Ala Ile Ile His Glu Glu Lys Ala Leu Thr Met Glu Val Val Arg Gln
85 90 95

Val Lys Gly Asp Leu Ala Phe Leu Asn Phe Gln Asn Asn Leu Gln Gln
100 105 110

Thr Pro Leu His Leu Ala Val Ile Thr Asn Gln Pro Glu Ile Ala Glu
115 120 125

Ala Leu Leu Glu Ala Gly Cys Asp Pro Glu Leu Arg Asp Phe Arg Gly
130 135 140

Asn Thr Pro Leu His Leu Ala Cys Glu Gln Gly Cys Leu Ala Ser Val
145 150 155 160

Gly Val Leu Thr Gln Pro Arg Gly Thr Gln His Leu His Ser Ile Leu
165 170 175

Gln Ala Thr Asn Tyr Asn Gly His Thr Cys Leu His Leu Ala Ser Ile
180 185 190

His Gly Tyr Leu Gly Ile Val Glu Leu Leu Val Ser Leu Gly Ala Asp
195 200 205

Val Asn Ala Gln Glu Pro Cys Asn Gly Arg Thr Ala Leu His Leu Ala

210 215 220

Val Asp Leu Gln Asn Pro Asp Leu Val Ser Leu Leu Leu Lys Cys Gly
225 230 235 240

Ala Asp Val Asn Arg Val Thr Tyr Gln Gly Tyr Ser Pro Tyr Gln Leu
245 250 255

Thr Trp Gly Arg Pro Ser Thr Arg Ile Gln Gln Gln Leu Gly Gln Leu
260 265 270

Thr Leu Glu Asn Leu Gln Met Leu Pro Glu Ser Glu Asp Glu Glu Ser
275 280 285

Tyr Asp Thr Glu Ser Glu Phe Thr Glu Asp Glu Leu Pro Tyr Asp Asp
290 295 300

Cys Val Leu Gly Gly Gln Arg Leu Thr Leu
305 310

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2011 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(B) LOCATION: 1..2011
(D) OTHER INFORMATION: /note= "NIM1 cDNA sequence"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 43..1824
(D) OTHER INFORMATION: /product= "NIM1 protein"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
GATCTCTTTA ATTTGTGAAT TTCAATTCAT CGGAACCTGT TG ATG GAC ACC ACC        54
                                                  Met Asp Thr Thr
                                                    1

ATT GAT GGA TTC GCC GAT TCT TAT GAA ATC AGC AGC ACT AGT TTC GTC      102
Ile Asp Gly Phe Ala Asp Ser Tyr Glu Ile Ser Ser Thr Ser Phe Val
  5                  10                  15                  20

GCT ACC GAT AAC ACC GAC TCC TCT ATT GTT TAT CTG GCC GCC GAA CAA      150
Ala Thr Asp Asn Thr Asp Ser Ser Ile Val Tyr Leu Ala Ala Glu Gln
                  25                  30                  35

GTA CTC ACC GGA CCT GAT GTA TCT GCT CTG CAA TTG CTC TCC AAC AGC      198
Val Leu Thr Gly Pro Asp Val Ser Ala Leu Gln Leu Leu Ser Asn Ser
              40                  45                  50

TTC GAA TCC GTC TTT GAC TCG CCG GAT GAT TTC TAC AGC GAC GCT AAG      246
Phe Glu Ser Val Phe Asp Ser Pro Asp Asp Phe Tyr Ser Asp Ala Lys
          55                  60                  65

CTT GTT CTC TCC GAC GGC CGG GAA GTT TCT TTC CAC CGG TGC GTT TTG      294
Leu Val Leu Ser Asp Gly Arg Glu Val Ser Phe His Arg Cys Val Leu
     70                  75                  80

TCA GCG AGA AGC TCT TTC TTC AAG AGC GCT TTA GCC GCC GCT AAG AAG      342
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```
Ser Ala Arg Ser Ser Phe Phe Lys Ser Ala Leu Ala Ala Ala Lys Lys
 85                  90                  95                 100

GAG AAA GAC TCC AAC AAC ACC GCC GCC GTG AAG CTC GAG CTT AAG GAG      390
Glu Lys Asp Ser Asn Asn Thr Ala Ala Val Lys Leu Glu Leu Lys Glu
                105                 110                 115

ATT GCC AAG GAT TAC GAA GTC GGT TTC GAT TCG GTT GTG ACT GTT TTG      438
Ile Ala Lys Asp Tyr Glu Val Gly Phe Asp Ser Val Val Thr Val Leu
            120                 125                 130

GCT TAT GTT TAC AGC AGC AGA GTG AGA CCG CCG CCT AAA GGA GTT TCT      486
Ala Tyr Val Tyr Ser Ser Arg Val Arg Pro Pro Pro Lys Gly Val Ser
        135                 140                 145

GAA TGC GCA GAC GAG AAT TGC TGC CAC GTG GCT TGC CGG CCG GCG GTG      534
Glu Cys Ala Asp Glu Asn Cys Cys His Val Ala Cys Arg Pro Ala Val
    150                 155                 160

GAT TTC ATG TTG GAG GTT CTC TAT TTG GCT TTC ATC TTC AAG ATC CCT      582
Asp Phe Met Leu Glu Val Leu Tyr Leu Ala Phe Ile Phe Lys Ile Pro
165                 170                 175                 180

GAA TTA ATT ACT CTC TAT CAG AGG CAC TTA TTG GAC GTT GTA GAC AAA      630
Glu Leu Ile Thr Leu Tyr Gln Arg His Leu Leu Asp Val Val Asp Lys
                185                 190                 195

GTT GTT ATA GAG GAC ACA TTG GTT ATA CTC AAG CTT GCT AAT ATA TGT      678
Val Val Ile Glu Asp Thr Leu Val Ile Leu Lys Leu Ala Asn Ile Cys
            200                 205                 210

GGT AAA GCT TGT ATG AAG CTA TTG GAT AGA TGT AAA GAG ATT ATT GTC      726
Gly Lys Ala Cys Met Lys Leu Leu Asp Arg Cys Lys Glu Ile Ile Val
        215                 220                 225

AAG TCT AAT GTA GAT ATG GTT AGT CTT GAA AAG TCA TTG CCG GAA GAG      774
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```
Lys Ser Asn Val Asp Met Val Ser Leu Glu Lys Ser Leu Pro Glu Glu
    230                 235                 240

CTT GTT AAA GAG ATA ATT GAT AGA CGT AAA GAG CTT GGT TTG GAG GTA      822
Leu Val Lys Glu Ile Ile Asp Arg Arg Lys Glu Leu Gly Leu Glu Val
245                 250                 255                 260

CCT AAA GTA AAG AAA CAT GTC TCG AAT GTA CAT AAG GCA CTT GAC TCG      870
Pro Lys Val Lys Lys His Val Ser Asn Val His Lys Ala Leu Asp Ser
                265                 270                 275

GAT GAT ATT GAG TTA GTC AAG TTG CTT TTG AAA GAG GAT CAC ACC AAT      918
Asp Asp Ile Glu Leu Val Lys Leu Leu Leu Lys Glu Asp His Thr Asn
            280                 285                 290

CTA GAT GAT GCG TGT GCT CTT CAT TTC GCT GTT GCA TAT TGC AAT GTG      966
Leu Asp Asp Ala Cys Ala Leu His Phe Ala Val Ala Tyr Cys Asn Val
        295                 300                 305

AAG ACC GCA ACA GAT CTT TTA AAA CTT GAT CTT GCC GAT GTC AAC CAT     1014
Lys Thr Ala Thr Asp Leu Leu Lys Leu Asp Leu Ala Asp Val Asn His
    310                 315                 320

AGG AAT CCG AGG GGA TAT ACG GTG CTT CAT GTT GCT GCG ATG CGG AAG     1062
Arg Asn Pro Arg Gly Tyr Thr Val Leu His Val Ala Ala Met Arg Lys
325                 330                 335                 340

GAG CCA CAA TTG ATA CTA TCT CTA TTG GAA AAA GGT GCA AGT GCA TCA     1110
Glu Pro Gln Leu Ile Leu Ser Leu Leu Glu Lys Gly Ala Ser Ala Ser
                345                 350                 355

GAA GCA ACT TTG GAA GGT AGA ACC GCA CTC ATG ATC GCA AAA CAA GCC     1158
Glu Ala Thr Leu Glu Gly Arg Thr Ala Leu Met Ile Ala Lys Gln Ala
            360                 365                 370

ACT ATG GCG GTT GAA TGT AAT AAT ATC CCG GAG CAA TGC AAG CAT TCT     1206
```

```
Thr Met Ala Val Glu Cys Asn Asn Ile Pro Glu Gln Cys Lys His Ser
        375                 380                 385

CTC AAA GGC CGA CTA TGT GTA GAA ATA CTA GAG CAA GAA GAC AAA CGA      1254
Leu Lys Gly Arg Leu Cys Val Glu Ile Leu Glu Gln Glu Asp Lys Arg
    390                 395                 400

GAA CAA ATT CCT AGA GAT GTT CCT CCC TCT TTT GCA GTG GCG GCC GAT      1302
Glu Gln Ile Pro Arg Asp Val Pro Pro Ser Phe Ala Val Ala Ala Asp
405                 410                 415                 420

GAA TTG AAG ATG ACG CTG CTC GAT CTT GAA AAT AGA GTT GCA CTT GCT      1350
Glu Leu Lys Met Thr Leu Leu Asp Leu Glu Asn Arg Val Ala Leu Ala
                425                 430                 435

CAA CGT CTT TTT CCA ACG GAA GCA CAA GCT GCA ATG GAG ATC GCC GAA      1398
Gln Arg Leu Phe Pro Thr Glu Ala Gln Ala Ala Met Glu Ile Ala Glu
            440                 445                 450

ATG AAG GGA ACA TGT GAG TTC ATA GTG ACT AGC CTC GAG CCT GAC CGT      1446
Met Lys Gly Thr Cys Glu Phe Ile Val Thr Ser Leu Glu Pro Asp Arg
        455                 460                 465

CTC ACT GGT ACG AAG AGA ACA TCA CCG GGT GTA AAG ATA GCA CCT TTC      1494
Leu Thr Gly Thr Lys Arg Thr Ser Pro Gly Val Lys Ile Ala Pro Phe
    470                 475                 480

AGA ATC CTA GAA GAG CAT CAA AGT AGA CTA AAA GCG CTT TCT AAA ACC      1542
Arg Ile Leu Glu Glu His Gln Ser Arg Leu Lys Ala Leu Ser Lys Thr
485                 490                 495                 500

GTG GAA CTC GGG AAA CGA TTC TTC CCG CGC TGT TCG GCA GTG CTC GAC      1590
Val Glu Leu Gly Lys Arg Phe Phe Pro Arg Cys Ser Ala Val Leu Asp
                505                 510                 515

CAG ATT ATG AAC TGT GAG GAC TTG ACT CAA CTG GCT TGC GGA GAA GAC      1638
```

```
Gln Ile Met Asn Cys Glu Asp Leu Thr Gln Leu Ala Cys Gly Glu Asp
            520                 525                 530

GAC ACT GCT GAG AAA CGA CTA CAA AAG AAG CAA AGG TAC ATG GAA ATA      1686
Asp Thr Ala Glu Lys Arg Leu Gln Lys Lys Gln Arg Tyr Met Glu Ile
        535                 540                 545

CAA GAG ACA CTA AAG AAG GCC TTT AGT GAG GAC AAT TTG GAA TTA GGA      1734
Gln Glu Thr Leu Lys Lys Ala Phe Ser Glu Asp Asn Leu Glu Leu Gly
    550                 555                 560

AAT TTG TCC CTG ACA GAT TCG ACT TCT TCC ACA TCG AAA TCA ACC GGT      1782
Asn Leu Ser Leu Thr Asp Ser Thr Ser Ser Thr Ser Lys Ser Thr Gly
565                 570                 575                 580

GGA AAG AGG TCT AAC CGT AAA CTC TCT CAT CGT CGT CGG TGA              1824
Gly Lys Arg Ser Asn Arg Lys Leu Ser His Arg Arg Arg  *
                585                 590

GACTCTTGCC TCTTAGTGTA ATTTTTGCTG TACCATATAA TTCTGTTTTC ATGATGACTG    1884

TAACTGTTTA TGTCTATCGT TGGCGTCATA TAGTTTCGCT CTTCGTTTTG CATCCTGTGT    1944

ATTATTGCTG CAGGTGTGCT TCAAACAAAT GTTGTAACAA TTTGAACCAA TGGTATACAG    2004

ATTTGTA                                                              2011
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2011 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 43..1824
(D) OTHER INFORMATION: /product= "altered form of NIM1" /note= "Serine residues at amino acid positions 55 and 59 in wild-type NIM1 gene product have been changed to Alanine residues."

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 205..217
(D) OTHER INFORMATION: /note= "nucleotides 205 and 217 changed from T's to G's compared to wild-type sequence."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
GATCTCTTTA ATTTGTGAAT TCAATTCAT CGGAACCTGT TG ATG GAC ACC ACC        54
                                                 Met Asp Thr Thr
                                                   1

ATT GAT GGA TTC GCC GAT TCT TAT GAA ATC AGC AGC ACT AGT TTC GTC      102
Ile Asp Gly Phe Ala Asp Ser Tyr Glu Ile Ser Ser Thr Ser Phe Val
  5                  10                  15                  20

GCT ACC GAT AAC ACC GAC TCC TCT ATT GTT TAT CTG GCC GCC GAA CAA      150
Ala Thr Asp Asn Thr Asp Ser Ser Ile Val Tyr Leu Ala Ala Glu Gln
                  25                  30                  35

GTA CTC ACC GGA CCT GAT GTA TCT GCT CTG CAA TTG CTC TCC AAC AGC      198
Val Leu Thr Gly Pro Asp Val Ser Ala Leu Gln Leu Leu Ser Asn Ser
             40                  45                  50
```

```
TTC GAA GCC GTC TTT GAC GCG CCG GAT GAT TTC TAC AGC GAC GCT AAG      246
Phe Glu Ala Val Phe Asp Ala Pro Asp Asp Phe Tyr Ser Asp Ala Lys
          55                  60                  65

CTT GTT CTC TCC GAC GGC CGG GAA GTT TCT TTC CAC CGG TGC GTT TTG      294
Leu Val Leu Ser Asp Gly Arg Glu Val Ser Phe His Arg Cys Val Leu
      70                  75                  80

TCA GCG AGA AGC TCT TTC TTC AAG AGC GCT TTA GCC GCC GCT AAG AAG      342
Ser Ala Arg Ser Ser Phe Phe Lys Ser Ala Leu Ala Ala Ala Lys Lys
 85                  90                  95                  100

GAG AAA GAC TCC AAC AAC ACC GCC GCC GTG AAG CTC GAG CTT AAG GAG      390
Glu Lys Asp Ser Asn Asn Thr Ala Ala Val Lys Leu Glu Leu Lys Glu
                105                 110                 115

ATT GCC AAG GAT TAC GAA GTC GGT TTC GAT TCG GTT GTG ACT GTT TTG      438
Ile Ala Lys Asp Tyr Glu Val Gly Phe Asp Ser Val Val Thr Val Leu
            120                 125                 130

GCT TAT GTT TAC AGC AGC AGA GTG AGA CCG CCG CCT AAA GGA GTT TCT      486
Ala Tyr Val Tyr Ser Ser Arg Val Arg Pro Pro Pro Lys Gly Val Ser
        135                 140                 145

GAA TGC GCA GAC GAG AAT TGC TGC CAC GTG GCT TGC CGG CCG GCG GTG      534
Glu Cys Ala Asp Glu Asn Cys Cys His Val Ala Cys Arg Pro Ala Val
    150                 155                 160

GAT TTC ATG TTG GAG GTT CTC TAT TTG GCT TTC ATC TTC AAG ATC CCT      582
Asp Phe Met Leu Glu Val Leu Tyr Leu Ala Phe Ile Phe Lys Ile Pro
165                 170                 175                 180

GAA TTA ATT ACT CTC TAT CAG AGG CAC TTA TTG GAC GTT GTA GAC AAA      630
Glu Leu Ile Thr Leu Tyr Gln Arg His Leu Leu Asp Val Val Asp Lys
                185                 190                 195
```

GTT GTT ATA GAG GAC ACA TTG GTT ATA CTC AAG CTT GCT AAT ATA TGT 678
Val Val Ile Glu Asp Thr Leu Val Ile Leu Lys Leu Ala Asn Ile Cys
200 205 210

GGT AAA GCT TGT ATG AAG CTA TTG GAT AGA TGT AAA GAG ATT ATT GTC 726
Gly Lys Ala Cys Met Lys Leu Leu Asp Arg Cys Lys Glu Ile Ile Val
215 220 225

AAG TCT AAT GTA GAT ATG GTT AGT CTT GAA AAG TCA TTG CCG GAA GAG 774
Lys Ser Asn Val Asp Met Val Ser Leu Glu Lys Ser Leu Pro Glu Glu
230 235 240

CTT GTT AAA GAG ATA ATT GAT AGA CGT AAA GAG CTT GGT TTG GAG GTA 822
Leu Val Lys Glu Ile Ile Asp Arg Arg Lys Glu Leu Gly Leu Glu Val
245 250 255 260

CCT AAA GTA AAG AAA CAT GTC TCG AAT GTA CAT AAG GCA CTT GAC TCG 870
Pro Lys Val Lys Lys His Val Ser Asn Val His Lys Ala Leu Asp Ser
265 270 275

GAT GAT ATT GAG TTA GTC AAG TTG CTT TTG AAA GAG GAT CAC ACC AAT 918
Asp Asp Ile Glu Leu Val Lys Leu Leu Leu Lys Glu Asp His Thr Asn
280 285 290

CTA GAT GAT GCG TGT GCT CTT CAT TTC GCT GTT GCA TAT TGC AAT GTG 966
Leu Asp Asp Ala Cys Ala Leu His Phe Ala Val Ala Tyr Cys Asn Val
295 300 305

AAG ACC GCA ACA GAT CTT TTA AAA CTT GAT CTT GCC GAT GTC AAC CAT 1014
Lys Thr Ala Thr Asp Leu Leu Lys Leu Asp Leu Ala Asp Val Asn His
310 315 320

AGG AAT CCG AGG GGA TAT ACG GTG CTT CAT GTT GCT GCG ATG CGG AAG 1062
Arg Asn Pro Arg Gly Tyr Thr Val Leu His Val Ala Ala Met Arg Lys
325 330 335 340

```
GAG CCA CAA TTG ATA CTA TCT CTA TTG GAA AAA GGT GCA AGT GCA TCA      1110
Glu Pro Gln Leu Ile Leu Ser Leu Leu Glu Lys Gly Ala Ser Ala Ser
                345                 350                 355

GAA GCA ACT TTG GAA GGT AGA ACC GCA CTC ATG ATC GCA AAA CAA GCC      1158
Glu Ala Thr Leu Glu Gly Arg Thr Ala Leu Met Ile Ala Lys Gln Ala
            360                 365                 370

ACT ATG GCG GTT GAA TGT AAT AAT ATC CCG GAG CAA TGC AAG CAT TCT      1206
Thr Met Ala Val Glu Cys Asn Asn Ile Pro Glu Gln Cys Lys His Ser
        375                 380                 385

CTC AAA GGC CGA CTA TGT GTA GAA ATA CTA GAG CAA GAA GAC AAA CGA      1254
Leu Lys Gly Arg Leu Cys Val Glu Ile Leu Glu Gln Glu Asp Lys Arg
    390                 395                 400

GAA CAA ATT CCT AGA GAT GTT CCT CCC TCT TTT GCA GTG GCG GCC GAT      1302
Glu Gln Ile Pro Arg Asp Val Pro Pro Ser Phe Ala Val Ala Ala Asp
405                 410                 415                 420

GAA TTG AAG ATG ACG CTG CTC GAT CTT GAA AAT AGA GTT GCA CTT GCT      1350
Glu Leu Lys Met Thr Leu Leu Asp Leu Glu Asn Arg Val Ala Leu Ala
                425                 430                 435

CAA CGT CTT TTT CCA ACG GAA GCA CAA GCT GCA ATG GAG ATC GCC GAA      1398
Gln Arg Leu Phe Pro Thr Glu Ala Gln Ala Ala Met Glu Ile Ala Glu
            440                 445                 450

ATG AAG GGA ACA TGT GAG TTC ATA GTG ACT AGC CTC GAG CCT GAC CGT      1446
Met Lys Gly Thr Cys Glu Phe Ile Val Thr Ser Leu Glu Pro Asp Arg
        455                 460                 465

CTC ACT GGT ACG AAG AGA ACA TCA CCG GGT GTA AAG ATA GCA CCT TTC      1494
Leu Thr Gly Thr Lys Arg Thr Ser Pro Gly Val Lys Ile Ala Pro Phe
    470                 475                 480
```

```
AGA ATC CTA GAA GAG CAT CAA AGT AGA CTA AAA GCG CTT TCT AAA ACC      1542
Arg Ile Leu Glu Glu His Gln Ser Arg Leu Lys Ala Leu Ser Lys Thr
485                 490                 495                 500

GTG GAA CTC GGG AAA CGA TTC TTC CCG CGC TGT TCG GCA GTG CTC GAC      1590
Val Glu Leu Gly Lys Arg Phe Phe Pro Arg Cys Ser Ala Val Leu Asp
                505                 510                 515

CAG ATT ATG AAC TGT GAG GAC TTG ACT CAA CTG GCT TGC GGA GAA GAC      1638
Gln Ile Met Asn Cys Glu Asp Leu Thr Gln Leu Ala Cys Gly Glu Asp
            520                 525                 530

GAC ACT GCT GAG AAA CGA CTA CAA AAG AAG CAA AGG TAC ATG GAA ATA      1686
Asp Thr Ala Glu Lys Arg Leu Gln Lys Lys Gln Arg Tyr Met Glu Ile
        535                 540                 545

CAA GAG ACA CTA AAG AAG GCC TTT AGT GAG GAC AAT TTG GAA TTA GGA      1734
Gln Glu Thr Leu Lys Lys Ala Phe Ser Glu Asp Asn Leu Glu Leu Gly
    550                 555                 560

AAT TTG TCC CTG ACA GAT TCG ACT TCT TCC ACA TCG AAA TCA ACC GGT      1782
Asn Leu Ser Leu Thr Asp Ser Thr Ser Ser Thr Ser Lys Ser Thr Gly
565                 570                 575                 580

GGA AAG AGG TCT AAC CGT AAA CTC TCT CAT CGT CGT CGG TGA              1824
Gly Lys Arg Ser Asn Arg Lys Leu Ser His Arg Arg Arg  *
                585                 590

GACTCTTGCC TCTTAGTGTA ATTTTTGCTG TACCATATAA TTCTGTTTTC ATGATGACTG    1884

TAACTGTTTA TGTCTATCGT TGGCGTCATA TAGTTTCGCT CTTCGTTTTG CATCCTGTGT    1944

ATTATTGCTG CAGGTGTGCT TCAAACAAAT GTTGTAACAA TTTGAACCAA TGGTATACAG    2004

ATTTGTA                                                              2011
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 594 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Asp Thr Thr Ile Asp Gly Phe Ala Asp Ser Tyr Glu Ile Ser Ser
1 5 10 15

Thr Ser Phe Val Ala Thr Asp Asn Thr Asp Ser Ser Ile Val Tyr Leu
20 25 30

Ala Ala Glu Gln Val Leu Thr Gly Pro Asp Val Ser Ala Leu Gln Leu
35 40 45

Leu Ser Asn Ser Phe Glu Ala Val Phe Asp Ala Pro Asp Asp Phe Tyr
50 55 60

Ser Asp Ala Lys Leu Val Leu Ser Asp Gly Arg Glu Val Ser Phe His
65 70 75 80

Arg Cys Val Leu Ser Ala Arg Ser Ser Phe Phe Lys Ser Ala Leu Ala
85 90 95

Ala Ala Lys Lys Glu Lys Asp Ser Asn Asn Thr Ala Ala Val Lys Leu
100 105 110

Glu Leu Lys Glu Ile Ala Lys Asp Tyr Glu Val Gly Phe Asp Ser Val
115 120 125

Val Thr Val Leu Ala Tyr Val Tyr Ser Ser Arg Val Arg Pro Pro Pro
130 135 140

Lys Gly Val Ser Glu Cys Ala Asp Glu Asn Cys Cys His Val Ala Cys
145 150 155 160

Arg Pro Ala Val Asp Phe Met Leu Glu Val Leu Tyr Leu Ala Phe Ile
165 170 175

Phe Lys Ile Pro Glu Leu Ile Thr Leu Tyr Gln Arg His Leu Leu Asp
180 185 190

Val Val Asp Lys Val Val Ile Glu Asp Thr Leu Val Ile Leu Lys Leu
195 200 205

Ala Asn Ile Cys Gly Lys Ala Cys Met Lys Leu Leu Asp Arg Cys Lys
210 215 220

Glu Ile Ile Val Lys Ser Asn Val Asp Met Val Ser Leu Glu Lys Ser
225 230 235 240

Leu Pro Glu Glu Leu Val Lys Glu Ile Ile Asp Arg Arg Lys Glu Leu
245 250 255

Gly Leu Glu Val Pro Lys Val Lys Lys His Val Ser Asn Val His Lys
260 265 270

Ala Leu Asp Ser Asp Asp Ile Glu Leu Val Lys Leu Leu Leu Lys Glu
275 280 285

Asp His Thr Asn Leu Asp Asp Ala Cys Ala Leu His Phe Ala Val Ala
290 295 300

Tyr Cys Asn Val Lys Thr Ala Thr Asp Leu Leu Lys Leu Asp Leu Ala
305 310 315 320

```
Asp Val Asn His Arg Asn Pro Arg Gly Tyr Thr Val Leu His Val Ala
                325                 330                 335

Ala Met Arg Lys Glu Pro Gln Leu Ile Leu Ser Leu Leu Glu Lys Gly
            340                 345                 350

Ala Ser Ala Ser Glu Ala Thr Leu Glu Gly Arg Thr Ala Leu Met Ile
        355                 360                 365

Ala Lys Gln Ala Thr Met Ala Val Glu Cys Asn Asn Ile Pro Glu Gln
    370                 375                 380

Cys Lys His Ser Leu Lys Gly Arg Leu Cys Val Glu Ile Leu Glu Gln
385                 390                 395                 400

Glu Asp Lys Arg Glu Gln Ile Pro Arg Asp Val Pro Pro Ser Phe Ala
                405                 410                 415

Val Ala Ala Asp Glu Leu Lys Met Thr Leu Leu Asp Leu Glu Asn Arg
            420                 425                 430

Val Ala Leu Ala Gln Arg Leu Phe Pro Thr Glu Ala Gln Ala Ala Met
        435                 440                 445

Glu Ile Ala Glu Met Lys Gly Thr Cys Glu Phe Ile Val Thr Ser Leu
    450                 455                 460

Glu Pro Asp Arg Leu Thr Gly Thr Lys Arg Thr Ser Pro Gly Val Lys
465                 470                 475                 480

Ile Ala Pro Phe Arg Ile Leu Glu Glu His Gln Ser Arg Leu Lys Ala
                485                 490                 495

Leu Ser Lys Thr Val Glu Leu Gly Lys Arg Phe Phe Pro Arg Cys Ser
            500                 505                 510
```

Ala Val Leu Asp Gln Ile Met Asn Cys Glu Asp Leu Thr Gln Leu Ala
515 520 525

Cys Gly Glu Asp Asp Thr Ala Glu Lys Arg Leu Gln Lys Lys Gln Arg
530 535 540

Tyr Met Glu Ile Gln Glu Thr Leu Lys Lys Ala Phe Ser Glu Asp Asn
545 550 555 560

Leu Glu Leu Gly Asn Leu Ser Leu Thr Asp Ser Thr Ser Ser Thr Ser
565 570 575

Lys Ser Thr Gly Gly Lys Arg Ser Asn Arg Lys Leu Ser His Arg Arg
580 585 590

Arg *

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1597 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1410
(D) OTHER INFORMATION: /product= "Altered form of NIM1"
/note= "N-terminal deletion compared to wild-type NIM1
sequence."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
ATG GAT TCG GTT GTG ACT GTT TTG GCT TAT GTT TAC AGC AGC AGA GTG        48
Met Asp Ser Val Val Thr Val Leu Ala Tyr Val Tyr Ser Ser Arg Val
 1               5                  10                  15

AGA CCG CCG CCT AAA GGA GTT TCT GAA TGC GCA GAC GAG AAT TGC TGC        96
Arg Pro Pro Pro Lys Gly Val Ser Glu Cys Ala Asp Glu Asn Cys Cys
            20                  25                  30

CAC GTG GCT TGC CGG CCG GCG GTG GAT TTC ATG TTG GAG GTT CTC TAT       144
His Val Ala Cys Arg Pro Ala Val Asp Phe Met Leu Glu Val Leu Tyr
        35                  40                  45

TTG GCT TTC ATC TTC AAG ATC CCT GAA TTA ATT ACT CTC TAT CAG AGG       192
Leu Ala Phe Ile Phe Lys Ile Pro Glu Leu Ile Thr Leu Tyr Gln Arg
    50                  55                  60

CAC TTA TTG GAC GTT GTA GAC AAA GTT GTT ATA GAG GAC ACA TTG GTT       240
His Leu Leu Asp Val Val Asp Lys Val Val Ile Glu Asp Thr Leu Val
65                  70                  75                  80

ATA CTC AAG CTT GCT AAT ATA TGT GGT AAA GCT TGT ATG AAG CTA TTG       288
Ile Leu Lys Leu Ala Asn Ile Cys Gly Lys Ala Cys Met Lys Leu Leu
                85                  90                  95

GAT AGA TGT AAA GAG ATT ATT GTC AAG TCT AAT GTA GAT ATG GTT AGT       336
Asp Arg Cys Lys Glu Ile Ile Val Lys Ser Asn Val Asp Met Val Ser
        100                 105                 110

CTT GAA AAG TCA TTG CCG GAA GAG CTT GTT AAA GAG ATA ATT GAT AGA       384
Leu Glu Lys Ser Leu Pro Glu Glu Leu Val Lys Glu Ile Ile Asp Arg
    115                 120                 125

CGT AAA GAG CTT GGT TTG GAG GTA CCT AAA GTA AAG AAA CAT GTC TCG       432
```

```
Arg Lys Glu Leu Gly Leu Glu Val Pro Lys Val Lys Lys His Val Ser
    130                 135                 140

AAT GTA CAT AAG GCA CTT GAC TCG GAT GAT ATT GAG TTA GTC AAG TTG      480
Asn Val His Lys Ala Leu Asp Ser Asp Asp Ile Glu Leu Val Lys Leu
145                 150                 155                 160

CTT TTG AAA GAG GAT CAC ACC AAT CTA GAT GAT GCG TGT GCT CTT CAT      528
Leu Leu Lys Glu Asp His Thr Asn Leu Asp Asp Ala Cys Ala Leu His
                165                 170                 175

TTC GCT GTT GCA TAT TGC AAT GTG AAG ACC GCA ACA GAT CTT TTA AAA      576
Phe Ala Val Ala Tyr Cys Asn Val Lys Thr Ala Thr Asp Leu Leu Lys
            180                 185                 190

CTT GAT CTT GCC GAT GTC AAC CAT AGG AAT CCG AGG GGA TAT ACG GTG      624
Leu Asp Leu Ala Asp Val Asn His Arg Asn Pro Arg Gly Tyr Thr Val
        195                 200                 205

CTT CAT GTT GCT GCG ATG CGG AAG GAG CCA CAA TTG ATA CTA TCT CTA      672
Leu His Val Ala Ala Met Arg Lys Glu Pro Gln Leu Ile Leu Ser Leu
    210                 215                 220

TTG GAA AAA GGT GCA AGT GCA TCA GAA GCA ACT TTG GAA GGT AGA ACC      720
Leu Glu Lys Gly Ala Ser Ala Ser Glu Ala Thr Leu Glu Gly Arg Thr
225                 230                 235                 240

GCA CTC ATG ATC GCA AAA CAA GCC ACT ATG GCG GTT GAA TGT AAT AAT      768
Ala Leu Met Ile Ala Lys Gln Ala Thr Met Ala Val Glu Cys Asn Asn
                245                 250                 255

ATC CCG GAG CAA TGC AAG CAT TCT CTC AAA GGC CGA CTA TGT GTA GAA      816
Ile Pro Glu Gln Cys Lys His Ser Leu Lys Gly Arg Leu Cys Val Glu
            260                 265                 270

ATA CTA GAG CAA GAA GAC AAA CGA GAA CAA ATT CCT AGA GAT GTT CCT      864
```

```
Ile Leu Glu Gln Glu Asp Lys Arg Glu Gln Ile Pro Arg Asp Val Pro
        275                 280                 285

CCC TCT TTT GCA GTG GCG GCC GAT GAA TTG AAG ATG ACG CTG CTC GAT      912
Pro Ser Phe Ala Val Ala Ala Asp Glu Leu Lys Met Thr Leu Leu Asp
    290                 295                 300

CTT GAA AAT AGA GTT GCA CTT GCT CAA CGT CTT TTT CCA ACG GAA GCA      960
Leu Glu Asn Arg Val Ala Leu Ala Gln Arg Leu Phe Pro Thr Glu Ala
305                 310                 315                 320

CAA GCT GCA ATG GAG ATC GCC GAA ATG AAG GGA ACA TGT GAG TTC ATA     1008
Gln Ala Ala Met Glu Ile Ala Glu Met Lys Gly Thr Cys Glu Phe Ile
                325                 330                 335

GTG ACT AGC CTC GAG CCT GAC CGT CTC ACT GGT ACG AAG AGA ACA TCA     1056
Val Thr Ser Leu Glu Pro Asp Arg Leu Thr Gly Thr Lys Arg Thr Ser
            340                 345                 350

CCG GGT GTA AAG ATA GCA CCT TTC AGA ATC CTA GAA GAG CAT CAA AGT     1104
Pro Gly Val Lys Ile Ala Pro Phe Arg Ile Leu Glu Glu His Gln Ser
        355                 360                 365

AGA CTA AAA GCG CTT TCT AAA ACC GTG GAA CTC GGG AAA CGA TTC TTC     1152
Arg Leu Lys Ala Leu Ser Lys Thr Val Glu Leu Gly Lys Arg Phe Phe
    370                 375                 380

CCG CGC TGT TCG GCA GTG CTC GAC CAG ATT ATG AAC TGT GAG GAC TTG     1200
Pro Arg Cys Ser Ala Val Leu Asp Gln Ile Met Asn Cys Glu Asp Leu
385                 390                 395                 400

ACT CAA CTG GCT TGC GGA GAA GAC GAC ACT GCT GAG AAA CGA CTA CAA     1248
Thr Gln Leu Ala Cys Gly Glu Asp Asp Thr Ala Glu Lys Arg Leu Gln
                405                 410                 415

AAG AAG CAA AGG TAC ATG GAA ATA CAA GAG ACA CTA AAG AAG GCC TTT     1296
```

```
Lys Lys Gln Arg Tyr Met Glu Ile Gln Glu Thr Leu Lys Lys Ala Phe
            420                 425                 430

AGT GAG GAC AAT TTG GAA TTA GGA AAT TTG TCC CTG ACA GAT TCG ACT      1344
Ser Glu Asp Asn Leu Glu Leu Gly Asn Leu Ser Leu Thr Asp Ser Thr
        435                 440                 445

TCT TCC ACA TCG AAA TCA ACC GGT GGA AAG AGG TCT AAC CGT AAA CTC      1392
Ser Ser Thr Ser Lys Ser Thr Gly Gly Lys Arg Ser Asn Arg Lys Leu
    450                 455                 460

TCT CAT CGT CGT CGG TGA GACTCTTGCC TCTTAGTGTA ATTTTTGCTG             1440
Ser His Arg Arg Arg  *
465                 470

TACCATATAA TTCTGTTTTC ATGATGACTG TAACTGTTTA TGTCTATCGT TGGCGTCATA    1500

TAGTTTCGCT CTTCGTTTTG CATCCTGTGT ATTATTGCTG CAGGTGTGCT TCAAACAAAT    1560

GTTGTAACAA TTTGAACCAA TGGTATACAG ATTTGTA                             1597
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 470 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Met Asp Ser Val Val Thr Val Leu Ala Tyr Val Tyr Ser Ser Arg Val
 1               5                  10                  15
```

Arg Pro Pro Pro Lys Gly Val Ser Glu Cys Ala Asp Glu Asn Cys Cys
20 25 30

His Val Ala Cys Arg Pro Ala Val Asp Phe Met Leu Glu Val Leu Tyr
35 40 45

Leu Ala Phe Ile Phe Lys Ile Pro Glu Leu Ile Thr Leu Tyr Gln Arg
50 55 60

His Leu Leu Asp Val Val Asp Lys Val Val Ile Glu Asp Thr Leu Val
65 70 75 80

Ile Leu Lys Leu Ala Asn Ile Cys Gly Lys Ala Cys Met Lys Leu Leu
85 90 95

Asp Arg Cys Lys Glu Ile Ile Val Lys Ser Asn Val Asp Met Val Ser
100 105 110

Leu Glu Lys Ser Leu Pro Glu Glu Leu Val Lys Glu Ile Ile Asp Arg
115 120 125

Arg Lys Glu Leu Gly Leu Glu Val Pro Lys Val Lys Lys His Val Ser
130 135 140

Asn Val His Lys Ala Leu Asp Ser Asp Asp Ile Glu Leu Val Lys Leu
145 150 155 160

Leu Leu Lys Glu Asp His Thr Asn Leu Asp Asp Ala Cys Ala Leu His
165 170 175

Phe Ala Val Ala Tyr Cys Asn Val Lys Thr Ala Thr Asp Leu Leu Lys
180 185 190

Leu Asp Leu Ala Asp Val Asn His Arg Asn Pro Arg Gly Tyr Thr Val
195 200 205

```
Leu His Val Ala Ala Met Arg Lys Glu Pro Gln Leu Ile Leu Ser Leu
    210                 215                 220

Leu Glu Lys Gly Ala Ser Ala Ser Glu Ala Thr Leu Glu Gly Arg Thr
225                 230                 235                 240

Ala Leu Met Ile Ala Lys Gln Ala Thr Met Ala Val Glu Cys Asn Asn
                245                 250                 255

Ile Pro Glu Gln Cys Lys His Ser Leu Lys Gly Arg Leu Cys Val Glu
            260                 265                 270

Ile Leu Glu Gln Glu Asp Lys Arg Glu Gln Ile Pro Arg Asp Val Pro
        275                 280                 285

Pro Ser Phe Ala Val Ala Ala Asp Glu Leu Lys Met Thr Leu Leu Asp
    290                 295                 300

Leu Glu Asn Arg Val Ala Leu Ala Gln Arg Leu Phe Pro Thr Glu Ala
305                 310                 315                 320

Gln Ala Ala Met Glu Ile Ala Glu Met Lys Gly Thr Cys Glu Phe Ile
                325                 330                 335

Val Thr Ser Leu Glu Pro Asp Arg Leu Thr Gly Thr Lys Arg Thr Ser
            340                 345                 350

Pro Gly Val Lys Ile Ala Pro Phe Arg Ile Leu Glu Glu His Gln Ser
        355                 360                 365

Arg Leu Lys Ala Leu Ser Lys Thr Val Glu Leu Gly Lys Arg Phe Phe
    370                 375                 380

Pro Arg Cys Ser Ala Val Leu Asp Gln Ile Met Asn Cys Glu Asp Leu
385                 390                 395                 400
```

```
Thr Gln Leu Ala Cys Gly Glu Asp Asp Thr Ala Glu Lys Arg Leu Gln
                405                 410                 415

Lys Lys Gln Arg Tyr Met Glu Ile Gln Glu Thr Leu Lys Lys Ala Phe
            420                 425                 430

Ser Glu Asp Asn Leu Glu Leu Gly Asn Leu Ser Leu Thr Asp Ser Thr
        435                 440                 445

Ser Ser Thr Ser Lys Ser Thr Gly Gly Lys Arg Ser Asn Arg Lys Leu
    450                 455                 460

Ser His Arg Arg Arg  *
465                 470
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1608 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 43..1608
(D) OTHER INFORMATION: /product= "Altered form of NIM1"
/note= "C-terminal deletion compared to wild-type NIM1."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GATCTCTTTA ATTTGTGAAT TTCAATTCAT CGGAACCTGT TG ATG GAC ACC ACC 54

Met Asp Thr Thr
1

ATT GAT GGA TTC GCC GAT TCT TAT GAA ATC AGC AGC ACT AGT TTC GTC 102
Ile Asp Gly Phe Ala Asp Ser Tyr Glu Ile Ser Ser Thr Ser Phe Val
5 10 15 20

GCT ACC GAT AAC ACC GAC TCC TCT ATT GTT TAT CTG GCC GCC GAA CAA 150
Ala Thr Asp Asn Thr Asp Ser Ser Ile Val Tyr Leu Ala Ala Glu Gln
25 30 35

GTA CTC ACC GGA CCT GAT GTA TCT GCT CTG CAA TTG CTC TCC AAC AGC 198
Val Leu Thr Gly Pro Asp Val Ser Ala Leu Gln Leu Leu Ser Asn Ser
40 45 50

TTC GAA TCC GTC TTT GAC TCG CCG GAT GAT TTC TAC AGC GAC GCT AAG 246
Phe Glu Ser Val Phe Asp Ser Pro Asp Asp Phe Tyr Ser Asp Ala Lys
55 60 65

CTT GTT CTC TCC GAC GGC CGG GAA GTT TCT TTC CAC CGG TGC GTT TTG 294
Leu Val Leu Ser Asp Gly Arg Glu Val Ser Phe His Arg Cys Val Leu
70 75 80

TCA GCG AGA AGC TCT TTC TTC AAG AGC GCT TTA GCC GCC GCT AAG AAG 342
Ser Ala Arg Ser Ser Phe Phe Lys Ser Ala Leu Ala Ala Ala Lys Lys
85 90 95 100

GAG AAA GAC TCC AAC AAC ACC GCC GCC GTG AAG CTC GAG CTT AAG GAG 390
Glu Lys Asp Ser Asn Asn Thr Ala Ala Val Lys Leu Glu Leu Lys Glu
105 110 115

ATT GCC AAG GAT TAC GAA GTC GGT TTC GAT TCG GTT GTG ACT GTT TTG 438
Ile Ala Lys Asp Tyr Glu Val Gly Phe Asp Ser Val Val Thr Val Leu
120 125 130

GCT TAT GTT TAC AGC AGC AGA GTG AGA CCG CCG CCT AAA GGA GTT TCT 486

```
Ala Tyr Val Tyr Ser Ser Arg Val Arg Pro Pro Pro Lys Gly Val Ser
        135                 140                 145

GAA TGC GCA GAC GAG AAT TGC TGC CAC GTG GCT TGC CGG CCG GCG GTG      534
Glu Cys Ala Asp Glu Asn Cys Cys His Val Ala Cys Arg Pro Ala Val
    150                 155                 160

GAT TTC ATG TTG GAG GTT CTC TAT TTG GCT TTC ATC TTC AAG ATC CCT      582
Asp Phe Met Leu Glu Val Leu Tyr Leu Ala Phe Ile Phe Lys Ile Pro
165                 170                 175                 180

GAA TTA ATT ACT CTC TAT CAG AGG CAC TTA TTG GAC GTT GTA GAC AAA      630
Glu Leu Ile Thr Leu Tyr Gln Arg His Leu Leu Asp Val Val Asp Lys
                185                 190                 195

GTT GTT ATA GAG GAC ACA TTG GTT ATA CTC AAG CTT GCT AAT ATA TGT      678
Val Val Ile Glu Asp Thr Leu Val Ile Leu Lys Leu Ala Asn Ile Cys
            200                 205                 210

GGT AAA GCT TGT ATG AAG CTA TTG GAT AGA TGT AAA GAG ATT ATT GTC      726
Gly Lys Ala Cys Met Lys Leu Leu Asp Arg Cys Lys Glu Ile Ile Val
        215                 220                 225

AAG TCT AAT GTA GAT ATG GTT AGT CTT GAA AAG TCA TTG CCG GAA GAG      774
Lys Ser Asn Val Asp Met Val Ser Leu Glu Lys Ser Leu Pro Glu Glu
    230                 235                 240

CTT GTT AAA GAG ATA ATT GAT AGA CGT AAA GAG CTT GGT TTG GAG GTA      822
Leu Val Lys Glu Ile Ile Asp Arg Arg Lys Glu Leu Gly Leu Glu Val
245                 250                 255                 260

CCT AAA GTA AAG AAA CAT GTC TCG AAT GTA CAT AAG GCA CTT GAC TCG      870
Pro Lys Val Lys Lys His Val Ser Asn Val His Lys Ala Leu Asp Ser
                265                 270                 275

GAT GAT ATT GAG TTA GTC AAG TTG CTT TTG AAA GAG GAT CAC ACC AAT      918
```

```
Asp Asp Ile Glu Leu Val Lys Leu Leu Leu Lys Glu Asp His Thr Asn
            280                 285                 290

CTA GAT GAT GCG TGT GCT CTT CAT TTC GCT GTT GCA TAT TGC AAT GTG      966
Leu Asp Asp Ala Cys Ala Leu His Phe Ala Val Ala Tyr Cys Asn Val
        295                 300                 305

AAG ACC GCA ACA GAT CTT TTA AAA CTT GAT CTT GCC GAT GTC AAC CAT     1014
Lys Thr Ala Thr Asp Leu Leu Lys Leu Asp Leu Ala Asp Val Asn His
    310                 315                 320

AGG AAT CCG AGG GGA TAT ACG GTG CTT CAT GTT GCT GCG ATG CGG AAG     1062
Arg Asn Pro Arg Gly Tyr Thr Val Leu His Val Ala Ala Met Arg Lys
325                 330                 335                 340

GAG CCA CAA TTG ATA CTA TCT CTA TTG GAA AAA GGT GCA AGT GCA TCA     1110
Glu Pro Gln Leu Ile Leu Ser Leu Leu Glu Lys Gly Ala Ser Ala Ser
                345                 350                 355

GAA GCA ACT TTG GAA GGT AGA ACC GCA CTC ATG ATC GCA AAA CAA GCC     1158
Glu Ala Thr Leu Glu Gly Arg Thr Ala Leu Met Ile Ala Lys Gln Ala
            360                 365                 370

ACT ATG GCG GTT GAA TGT AAT AAT ATC CCG GAG CAA TGC AAG CAT TCT     1206
Thr Met Ala Val Glu Cys Asn Asn Ile Pro Glu Gln Cys Lys His Ser
        375                 380                 385

CTC AAA GGC CGA CTA TGT GTA GAA ATA CTA GAG CAA GAA GAC AAA CGA     1254
Leu Lys Gly Arg Leu Cys Val Glu Ile Leu Glu Gln Glu Asp Lys Arg
    390                 395                 400

GAA CAA ATT CCT AGA GAT GTT CCT CCC TCT TTT GCA GTG GCG GCC GAT     1302
Glu Gln Ile Pro Arg Asp Val Pro Pro Ser Phe Ala Val Ala Ala Asp
405                 410                 415                 420

GAA TTG AAG ATG ACG CTG CTC GAT CTT GAA AAT AGA GTT GCA CTT GCT     1350
```

```
Glu Leu Lys Met Thr Leu Leu Asp Leu Glu Asn Arg Val Ala Leu Ala
                425                 430                 435

CAA CGT CTT TTT CCA ACG GAA GCA CAA GCT GCA ATG GAG ATC GCC GAA      1398
Gln Arg Leu Phe Pro Thr Glu Ala Gln Ala Ala Met Glu Ile Ala Glu
            440                 445                 450

ATG AAG GGA ACA TGT GAG TTC ATA GTG ACT AGC CTC GAG CCT GAC CGT      1446
Met Lys Gly Thr Cys Glu Phe Ile Val Thr Ser Leu Glu Pro Asp Arg
        455                 460                 465

CTC ACT GGT ACG AAG AGA ACA TCA CCG GGT GTA AAG ATA GCA CCT TTC      1494
Leu Thr Gly Thr Lys Arg Thr Ser Pro Gly Val Lys Ile Ala Pro Phe
    470                 475                 480

AGA ATC CTA GAA GAG CAT CAA AGT AGA CTA AAA GCG CTT TCT AAA ACC      1542
Arg Ile Leu Glu Glu His Gln Ser Arg Leu Lys Ala Leu Ser Lys Thr
485                 490                 495                 500

GTG GAA CTC GGG AAA CGA TTC TTC CCG CGC TGT TCG GCA GTG CTC GAC      1590
Val Glu Leu Gly Lys Arg Phe Phe Pro Arg Cys Ser Ala Val Leu Asp
                505                 510                 515

CAG ATT ATG AAC TGT TGA                                              1608
Gln Ile Met Asn Cys  *
            520
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 522 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Met Asp Thr Thr Ile Asp Gly Phe Ala Asp Ser Tyr Glu Ile Ser Ser
1 5 10 15

Thr Ser Phe Val Ala Thr Asp Asn Thr Asp Ser Ser Ile Val Tyr Leu
20 25 30

Ala Ala Glu Gln Val Leu Thr Gly Pro Asp Val Ser Ala Leu Gln Leu
35 40 45

Leu Ser Asn Ser Phe Glu Ser Val Phe Asp Ser Pro Asp Asp Phe Tyr
50 55 60

Ser Asp Ala Lys Leu Val Leu Ser Asp Gly Arg Glu Val Ser Phe His
65 70 75 80

Arg Cys Val Leu Ser Ala Arg Ser Ser Phe Phe Lys Ser Ala Leu Ala
85 90 95

Ala Ala Lys Lys Glu Lys Asp Ser Asn Asn Thr Ala Ala Val Lys Leu
100 105 110

Glu Leu Lys Glu Ile Ala Lys Asp Tyr Glu Val Gly Phe Asp Ser Val
115 120 125

Val Thr Val Leu Ala Tyr Val Tyr Ser Ser Arg Val Arg Pro Pro Pro
130 135 140

Lys Gly Val Ser Glu Cys Ala Asp Glu Asn Cys Cys His Val Ala Cys
145 150 155 160

Arg Pro Ala Val Asp Phe Met Leu Glu Val Leu Tyr Leu Ala Phe Ile
165 170 175

```
Phe Lys Ile Pro Glu Leu Ile Thr Leu Tyr Gln Arg His Leu Leu Asp
            180                 185                 190

Val Val Asp Lys Val Val Ile Glu Asp Thr Leu Val Ile Leu Lys Leu
        195                 200                 205

Ala Asn Ile Cys Gly Lys Ala Cys Met Lys Leu Leu Asp Arg Cys Lys
    210                 215                 220

Glu Ile Ile Val Lys Ser Asn Val Asp Met Val Ser Leu Glu Lys Ser
225                 230                 235                 240

Leu Pro Glu Glu Leu Val Lys Glu Ile Ile Asp Arg Arg Lys Glu Leu
                245                 250                 255

Gly Leu Glu Val Pro Lys Val Lys Lys His Val Ser Asn Val His Lys
            260                 265                 270

Ala Leu Asp Ser Asp Asp Ile Glu Leu Val Lys Leu Leu Leu Lys Glu
        275                 280                 285

Asp His Thr Asn Leu Asp Asp Ala Cys Ala Leu His Phe Ala Val Ala
    290                 295                 300

Tyr Cys Asn Val Lys Thr Ala Thr Asp Leu Leu Lys Leu Asp Leu Ala
305                 310                 315                 320

Asp Val Asn His Arg Asn Pro Arg Gly Tyr Thr Val Leu His Val Ala
                325                 330                 335

Ala Met Arg Lys Glu Pro Gln Leu Ile Leu Ser Leu Leu Glu Lys Gly
            340                 345                 350

Ala Ser Ala Ser Glu Ala Thr Leu Glu Gly Arg Thr Ala Leu Met Ile
        355                 360                 365
```

Ala Lys Gln Ala Thr Met Ala Val Glu Cys Asn Asn Ile Pro Glu Gln
370 375 380

Cys Lys His Ser Leu Lys Gly Arg Leu Cys Val Glu Ile Leu Glu Gln
385 390 395 400

Glu Asp Lys Arg Glu Gln Ile Pro Arg Asp Val Pro Pro Ser Phe Ala
405 410 415

Val Ala Ala Asp Glu Leu Lys Met Thr Leu Leu Asp Leu Glu Asn Arg
420 425 430

Val Ala Leu Ala Gln Arg Leu Phe Pro Thr Glu Ala Gln Ala Ala Met
435 440 445

Glu Ile Ala Glu Met Lys Gly Thr Cys Glu Phe Ile Val Thr Ser Leu
450 455 460

Glu Pro Asp Arg Leu Thr Gly Thr Lys Arg Thr Ser Pro Gly Val Lys
465 470 475 480

Ile Ala Pro Phe Arg Ile Leu Glu Glu His Gln Ser Arg Leu Lys Ala
485 490 495

Leu Ser Lys Thr Val Glu Leu Gly Lys Arg Phe Phe Pro Arg Cys Ser
500 505 510

Ala Val Leu Asp Gln Ile Met Asn Cys *
515 520

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1194 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1194
(D) OTHER INFORMATION: /product= "Altered form of NIM1"
/note= "N-terminal/C-terminal chimera."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
ATG GAT TCG GTT GTG ACT GTT TTG GCT TAT GTT TAC AGC AGC AGA GTG      48
Met Asp Ser Val Val Thr Val Leu Ala Tyr Val Tyr Ser Ser Arg Val
 1               5                  10                  15

AGA CCG CCG CCT AAA GGA GTT TCT GAA TGC GCA GAC GAG AAT TGC TGC      96
Arg Pro Pro Pro Lys Gly Val Ser Glu Cys Ala Asp Glu Asn Cys Cys
            20                  25                  30

CAC GTG GCT TGC CGG CCG GCG GTG GAT TTC ATG TTG GAG GTT CTC TAT     144
His Val Ala Cys Arg Pro Ala Val Asp Phe Met Leu Glu Val Leu Tyr
        35                  40                  45

TTG GCT TTC ATC TTC AAG ATC CCT GAA TTA ATT ACT CTC TAT CAG AGG     192
Leu Ala Phe Ile Phe Lys Ile Pro Glu Leu Ile Thr Leu Tyr Gln Arg
    50                  55                  60

CAC TTA TTG GAC GTT GTA GAC AAA GTT GTT ATA GAG GAC ACA TTG GTT     240
His Leu Leu Asp Val Val Asp Lys Val Val Ile Glu Asp Thr Leu Val
65                  70                  75                  80

ATA CTC AAG CTT GCT AAT ATA TGT GGT AAA GCT TGT ATG AAG CTA TTG     288
Ile Leu Lys Leu Ala Asn Ile Cys Gly Lys Ala Cys Met Lys Leu Leu
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                    85                  90                  95

GAT AGA TGT AAA GAG ATT ATT GTC AAG TCT AAT GTA GAT ATG GTT AGT      336
Asp Arg Cys Lys Glu Ile Ile Val Lys Ser Asn Val Asp Met Val Ser
            100                 105                 110

CTT GAA AAG TCA TTG CCG GAA GAG CTT GTT AAA GAG ATA ATT GAT AGA      384
Leu Glu Lys Ser Leu Pro Glu Glu Leu Val Lys Glu Ile Ile Asp Arg
        115                 120                 125

CGT AAA GAG CTT GGT TTG GAG GTA CCT AAA GTA AAG AAA CAT GTC TCG      432
Arg Lys Glu Leu Gly Leu Glu Val Pro Lys Val Lys Lys His Val Ser
    130                 135                 140

AAT GTA CAT AAG GCA CTT GAC TCG GAT GAT ATT GAG TTA GTC AAG TTG      480
Asn Val His Lys Ala Leu Asp Ser Asp Asp Ile Glu Leu Val Lys Leu
145                 150                 155                 160

CTT TTG AAA GAG GAT CAC ACC AAT CTA GAT GAT GCG TGT GCT CTT CAT      528
Leu Leu Lys Glu Asp His Thr Asn Leu Asp Asp Ala Cys Ala Leu His
                165                 170                 175

TTC GCT GTT GCA TAT TGC AAT GTG AAG ACC GCA ACA GAT CTT TTA AAA      576
Phe Ala Val Ala Tyr Cys Asn Val Lys Thr Ala Thr Asp Leu Leu Lys
            180                 185                 190

CTT GAT CTT GCC GAT GTC AAC CAT AGG AAT CCG AGG GGA TAT ACG GTG      624
Leu Asp Leu Ala Asp Val Asn His Arg Asn Pro Arg Gly Tyr Thr Val
        195                 200                 205

CTT CAT GTT GCT GCG ATG CGG AAG GAG CCA CAA TTG ATA CTA TCT CTA      672
Leu His Val Ala Ala Met Arg Lys Glu Pro Gln Leu Ile Leu Ser Leu
    210                 215                 220

TTG GAA AAA GGT GCA AGT GCA TCA GAA GCA ACT TTG GAA GGT AGA ACC      720
Leu Glu Lys Gly Ala Ser Ala Ser Glu Ala Thr Leu Glu Gly Arg Thr
```

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225                 230                 235                 240

GCA CTC ATG ATC GCA AAA CAA GCC ACT ATG GCG GTT GAA TGT AAT AAT      768
Ala Leu Met Ile Ala Lys Gln Ala Thr Met Ala Val Glu Cys Asn Asn
                245                 250                 255

ATC CCG GAG CAA TGC AAG CAT TCT CTC AAA GGC CGA CTA TGT GTA GAA      816
Ile Pro Glu Gln Cys Lys His Ser Leu Lys Gly Arg Leu Cys Val Glu
            260                 265                 270

ATA CTA GAG CAA GAA GAC AAA CGA GAA CAA ATT CCT AGA GAT GTT CCT      864
Ile Leu Glu Gln Glu Asp Lys Arg Glu Gln Ile Pro Arg Asp Val Pro
        275                 280                 285

CCC TCT TTT GCA GTG GCG GCC GAT GAA TTG AAG ATG ACG CTG CTC GAT      912
Pro Ser Phe Ala Val Ala Ala Asp Glu Leu Lys Met Thr Leu Leu Asp
    290                 295                 300

CTT GAA AAT AGA GTT GCA CTT GCT CAA CGT CTT TTT CCA ACG GAA GCA      960
Leu Glu Asn Arg Val Ala Leu Ala Gln Arg Leu Phe Pro Thr Glu Ala
305                 310                 315                 320

CAA GCT GCA ATG GAG ATC GCC GAA ATG AAG GGA ACA TGT GAG TTC ATA     1008
Gln Ala Ala Met Glu Ile Ala Glu Met Lys Gly Thr Cys Glu Phe Ile
                325                 330                 335

GTG ACT AGC CTC GAG CCT GAC CGT CTC ACT GGT ACG AAG AGA ACA TCA     1056
Val Thr Ser Leu Glu Pro Asp Arg Leu Thr Gly Thr Lys Arg Thr Ser
            340                 345                 350

CCG GGT GTA AAG ATA GCA CCT TTC AGA ATC CTA GAA GAG CAT CAA AGT     1104
Pro Gly Val Lys Ile Ala Pro Phe Arg Ile Leu Glu Glu His Gln Ser
        355                 360                 365

AGA CTA AAA GCG CTT TCT AAA ACC GTG GAA CTC GGG AAA CGA TTC TTC     1152
Arg Leu Lys Ala Leu Ser Lys Thr Val Glu Leu Gly Lys Arg Phe Phe
```

370 375 380

CCG CGC TGT TCG GCA GTG CTC GAC CAG ATT ATG AAC TGT TGA 1194
Pro Arg Cys Ser Ala Val Leu Asp Gln Ile Met Asn Cys *
385 390 395

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 398 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Met Asp Ser Val Val Thr Val Leu Ala Tyr Val Tyr Ser Ser Arg Val
1 5 10 15

Arg Pro Pro Pro Lys Gly Val Ser Glu Cys Ala Asp Glu Asn Cys Cys
20 25 30

His Val Ala Cys Arg Pro Ala Val Asp Phe Met Leu Glu Val Leu Tyr
35 40 45

Leu Ala Phe Ile Phe Lys Ile Pro Glu Leu Ile Thr Leu Tyr Gln Arg
50 55 60

His Leu Leu Asp Val Val Asp Lys Val Val Ile Glu Asp Thr Leu Val
65 70 75 80

Ile Leu Lys Leu Ala Asn Ile Cys Gly Lys Ala Cys Met Lys Leu Leu
85 90 95

```
Asp Arg Cys Lys Glu Ile Ile Val Lys Ser Asn Val Asp Met Val Ser
            100                 105                 110

Leu Glu Lys Ser Leu Pro Glu Glu Leu Val Lys Glu Ile Ile Asp Arg
        115                 120                 125

Arg Lys Glu Leu Gly Leu Glu Val Pro Lys Val Lys Lys His Val Ser
    130                 135                 140

Asn Val His Lys Ala Leu Asp Ser Asp Asp Ile Glu Leu Val Lys Leu
145                 150                 155                 160

Leu Leu Lys Glu Asp His Thr Asn Leu Asp Asp Ala Cys Ala Leu His
                165                 170                 175

Phe Ala Val Ala Tyr Cys Asn Val Lys Thr Ala Thr Asp Leu Leu Lys
            180                 185                 190

Leu Asp Leu Ala Asp Val Asn His Arg Asn Pro Arg Gly Tyr Thr Val
        195                 200                 205

Leu His Val Ala Ala Met Arg Lys Glu Pro Gln Leu Ile Leu Ser Leu
    210                 215                 220

Leu Glu Lys Gly Ala Ser Ala Ser Glu Ala Thr Leu Glu Gly Arg Thr
225                 230                 235                 240

Ala Leu Met Ile Ala Lys Gln Ala Thr Met Ala Val Glu Cys Asn Asn
                245                 250                 255

Ile Pro Glu Gln Cys Lys His Ser Leu Lys Gly Arg Leu Cys Val Glu
            260                 265                 270

Ile Leu Glu Gln Glu Asp Lys Arg Glu Gln Ile Pro Arg Asp Val Pro
        275                 280                 285
```

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Pro Ser Phe Ala Val Ala Ala Asp Glu Leu Lys Met Thr Leu Leu Asp
    290                 295                 300

Leu Glu Asn Arg Val Ala Leu Ala Gln Arg Leu Phe Pro Thr Glu Ala
305                 310                 315                 320

Gln Ala Ala Met Glu Ile Ala Glu Met Lys Gly Thr Cys Glu Phe Ile
                325                 330                 335

Val Thr Ser Leu Glu Pro Asp Arg Leu Thr Gly Thr Lys Arg Thr Ser
            340                 345                 350

Pro Gly Val Lys Ile Ala Pro Phe Arg Ile Leu Glu Glu His Gln Ser
        355                 360                 365

Arg Leu Lys Ala Leu Ser Lys Thr Val Glu Leu Gly Lys Arg Phe Phe
    370                 375                 380

Pro Arg Cys Ser Ala Val Leu Asp Gln Ile Met Asn Cys  *
385                 390                 395
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 786 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
- (A) NAME/KEY: CDS
- (B) LOCATION: 1..786
- (D) OTHER INFORMATION: /product= "Altered form of NIM1"

/note= "Ankyrin domains of NIM1."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
ATG GAC TCC AAC AAC ACC GCC GCC GTG AAG CTC GAG CTT AAG GAG ATT        48
Met Asp Ser Asn Asn Thr Ala Ala Val Lys Leu Glu Leu Lys Glu Ile
 1               5                  10                  15

GCC AAG GAT TAC GAA GTC GGT TTC GAT TCG GTT GTG ACT GTT TTG GCT        96
Ala Lys Asp Tyr Glu Val Gly Phe Asp Ser Val Val Thr Val Leu Ala
            20                  25                  30

TAT GTT TAC AGC AGC AGA GTG AGA CCG CCG CCT AAA GGA GTT TCT GAA       144
Tyr Val Tyr Ser Ser Arg Val Arg Pro Pro Pro Lys Gly Val Ser Glu
        35                  40                  45

TGC GCA GAC GAG AAT TGC TGC CAC GTG GCT TGC CGG CCG GCG GTG GAT       192
Cys Ala Asp Glu Asn Cys Cys His Val Ala Cys Arg Pro Ala Val Asp
    50                  55                  60

TTC ATG TTG GAG GTT CTC TAT TTG GCT TTC ATC TTC AAG ATC CCT GAA       240
Phe Met Leu Glu Val Leu Tyr Leu Ala Phe Ile Phe Lys Ile Pro Glu
65                  70                  75                  80

TTA ATT ACT CTC TAT CAG AGG CAC TTA TTG GAC GTT GTA GAC AAA GTT       288
Leu Ile Thr Leu Tyr Gln Arg His Leu Leu Asp Val Val Asp Lys Val
                85                  90                  95

GTT ATA GAG GAC ACA TTG GTT ATA CTC AAG CTT GCT AAT ATA TGT GGT       336
Val Ile Glu Asp Thr Leu Val Ile Leu Lys Leu Ala Asn Ile Cys Gly
            100                 105                 110

AAA GCT TGT ATG AAG CTA TTG GAT AGA TGT AAA GAG ATT ATT GTC AAG       384
Lys Ala Cys Met Lys Leu Leu Asp Arg Cys Lys Glu Ile Ile Val Lys
        115                 120                 125
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TCT AAT GTA GAT ATG GTT AGT CTT GAA AAG TCA TTG CCG GAA GAG CTT        432
Ser Asn Val Asp Met Val Ser Leu Glu Lys Ser Leu Pro Glu Glu Leu
    130                 135                 140

GTT AAA GAG ATA ATT GAT AGA CGT AAA GAG CTT GGT TTG GAG GTA CCT        480
Val Lys Glu Ile Ile Asp Arg Arg Lys Glu Leu Gly Leu Glu Val Pro
145                 150                 155                 160

AAA GTA AAG AAA CAT GTC TCG AAT GTA CAT AAG GCA CTT GAC TCG GAT        528
Lys Val Lys Lys His Val Ser Asn Val His Lys Ala Leu Asp Ser Asp
                165                 170                 175

GAT ATT GAG TTA GTC AAG TTG CTT TTG AAA GAG GAT CAC ACC AAT CTA        576
Asp Ile Glu Leu Val Lys Leu Leu Leu Lys Glu Asp His Thr Asn Leu
            180                 185                 190

GAT GAT GCG TGT GCT CTT CAT TTC GCT GTT GCA TAT TGC AAT GTG AAG        624
Asp Asp Ala Cys Ala Leu His Phe Ala Val Ala Tyr Cys Asn Val Lys
        195                 200                 205

ACC GCA ACA GAT CTT TTA AAA CTT GAT CTT GCC GAT GTC AAC CAT AGG        672
Thr Ala Thr Asp Leu Leu Lys Leu Asp Leu Ala Asp Val Asn His Arg
    210                 215                 220

AAT CCG AGG GGA TAT ACG GTG CTT CAT GTT GCT GCG ATG CGG AAG GAG        720
Asn Pro Arg Gly Tyr Thr Val Leu His Val Ala Ala Met Arg Lys Glu
225                 230                 235                 240

CCA CAA TTG ATA CTA TCT CTA TTG GAA AAA GGT GCA AGT GCA TCA GAA        768
Pro Gln Leu Ile Leu Ser Leu Leu Glu Lys Gly Ala Ser Ala Ser Glu
                245                 250                 255

GCA ACT TTG GAA GGT TGA                                               786
Ala Thr Leu Glu Gly  *
            260
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 262 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Met Asp Ser Asn Asn Thr Ala Ala Val Lys Leu Glu Leu Lys Glu Ile
 1               5                  10                  15

Ala Lys Asp Tyr Glu Val Gly Phe Asp Ser Val Val Thr Val Leu Ala
            20                  25                  30

Tyr Val Tyr Ser Ser Arg Val Arg Pro Pro Pro Lys Gly Val Ser Glu
        35                  40                  45

Cys Ala Asp Glu Asn Cys Cys His Val Ala Cys Arg Pro Ala Val Asp
    50                  55                  60

Phe Met Leu Glu Val Leu Tyr Leu Ala Phe Ile Phe Lys Ile Pro Glu
65                  70                  75                  80

Leu Ile Thr Leu Tyr Gln Arg His Leu Leu Asp Val Val Asp Lys Val
                85                  90                  95

Val Ile Glu Asp Thr Leu Val Ile Leu Lys Leu Ala Asn Ile Cys Gly
            100                 105                 110

Lys Ala Cys Met Lys Leu Leu Asp Arg Cys Lys Glu Ile Ile Val Lys
        115                 120                 125
```

```
Ser Asn Val Asp Met Val Ser Leu Glu Lys Ser Leu Pro Glu Glu Leu
    130                 135                 140

Val Lys Glu Ile Ile Asp Arg Arg Lys Glu Leu Gly Leu Glu Val Pro
145                 150                 155                 160

Lys Val Lys Lys His Val Ser Asn Val His Lys Ala Leu Asp Ser Asp
                165                 170                 175

Asp Ile Glu Leu Val Lys Leu Leu Leu Lys Glu Asp His Thr Asn Leu
            180                 185                 190

Asp Asp Ala Cys Ala Leu His Phe Ala Val Ala Tyr Cys Asn Val Lys
        195                 200                 205

Thr Ala Thr Asp Leu Leu Lys Leu Asp Leu Ala Asp Val Asn His Arg
    210                 215                 220

Asn Pro Arg Gly Tyr Thr Val Leu His Val Ala Ala Met Arg Lys Glu
225                 230                 235                 240

Pro Gln Leu Ile Leu Ser Leu Leu Glu Lys Gly Ala Ser Ala Ser Glu
                245                 250                 255

Ala Thr Leu Glu Gly *
            260
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CAACAGCTTC GAAGCCGTCT TTGACGCGCC GGATG 35

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CATCCGGCGC GTCAAAGACG GCTTCGAAGC TGTTG 35

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGAATTCAAT GGATTCGGTT GTGACTGTTT TG 32

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GGAATTCTAC AAATCTGTAT ACCATTGG 28

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CGGAATTCGA TCTCTTTAAT TTGTGAATTT C 31

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GGAATTCTCA ACAGTTCATA ATCTGGTCG 29

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GGAATTCAAT GGACTCCAAC AACACCGCCG C 31

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GGAATTCTCA ACCTTCCAAA GTTGCTTCTG ATG 33

American Type Culture Collection

12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)231-5520 Telex: 898-055 ATCCNORTH • FAX: 301-770-2587

A-185
CIBA-101

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

RECEIVED
MAY 2 8 1996
CIBA-GEIGY
ABRU PATENT DEPT.

To: (Name and Address of Depositor or Attorney)

Ciba-Geigy Corporation
Attn: Leslie B. Friedrich
P.O. Box 12257
Research Triangle Park, NC 27709

Deposited on Behalf of: Ciba-Geigy Corporation

Id ntification Reference by Depositor:	ATCC Designation
Plasmid, BAC4	97543

The deposit was accompanied by: ___ a scientific description _ a proposed taxonomic description indicated above.

The deposit was received May 8, 1996 by this International Depository Authority and has been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strain for 30 years.

Th strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to r ceive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested May 17, 1996. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Barbara M. Hailey

Date: May 20, 1996

Barbara M. Hailey, Administrator, Patent Depository

cc: Andrea C. Walsh, Ph.D.

American Type Culture Collection

12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)231-5520 Telex: 898-055 ATCCNORTH • FAX: 301-770-2587

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Ciba-Geigy Corporation
Attn: Leslie B. Friedrich
P.O. Box 12257
Research Triangle Park, NC 27709

Deposited on Behalf of: Ciba-Geigy Corporation

Identification Reference by Depositor:	**ATCC Designation**
Plasmid P1-18	97606

The deposit was accompanied by: ___ a scientific description _ a proposed taxonomic description indicated above.

The deposit was received June 13, 1996 by this International Depository Authority and has been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested June 20, 1996. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Barbara M. Hailey

Barbara M. Hailey, Administrator, Patent Depository

Date: June 21, 1996

cc: Andrea C. Walsh, Ph.D.

American Type Culture Collection

12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)231-5520 Telex: 908-768 ATCCROVE • FAX: 301-816-4366

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Ciba-Geigy Corporation
Attention: Leslie B. Friedrich
P.O. Box 12257
Research Triangle Park, NC 27709

Deposited on Behalf of: Ciba-Geigy Corporation

Identification Reference by Depositor:	ATCC Designation
Cosmid, D7	97736

The deposit was accompanied by: ___ a scientific description ___ a proposed taxonomic description indicated above.

The deposit was received September 25, 1996 by this International Depository Authority and has been accepted.

AT YOUR REQUEST:

X We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested October 3, 1996. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Barbara M. Hailey

Barbara M. Hailey, Administrator, ATCC Patent Depository

Date: October 7, 1996

cc: Andrea C. Walsh, Ph.D.

What is claimed is:

1. A DNA molecule that encodes an altered form of a NIM1 protein.

2. The DNA molecule according to claim 1, that acts as a dominant-negative regulator of the SAR signal transduction pathway.

3. The DNA molecule according to claim 1, wherein said altered form of the NIM1 protein has alanines instead of serines in amino acid positions corresponding to positions 55 and 59 of SEQ ID NO:3.

4. The DNA molecule according to claim 3, wherein said altered form of the NIM1 protein comprises the amino acid sequence shown in SEQ ID NO:23.

5. The DNA molecule according to claim 4, wherein said DNA molecule comprises the nucleotide sequence shown in SEQ ID NO:22 and all DNA.

6. The DNA molecule according to claim 1, wherein the altered form of the *NIM1* protein is a truncated version of the *NIM1* gene product.

7. The DNA molecule according to claim 1, wherein said altered form of the NIM1 protein has an N-terminal truncation of amino acids corresponding approximately to amino acid positions 1-125 of SEQ ID NO:3.

8. The DNA molecule according to claim 7, wherein said altered form of the NIM1 protein comprises the amino acid sequence shown in SEQ ID NO:25.

9. The DNA molecule according to claim 8, wherein said DNA molecule comprises the nucleotide sequence shown in SEQ ID NO:24.

10. The DNA molecule according to claim 1, wherein said altered form of the NIM1 protein has a C-terminal truncation of amino acids corresponding approximately to amino acid positions 522-593 of SEQ ID NO:3.

11. The DNA molecule according to claim 22, wherein said altered form of the NIM1 protein comprises the amino acid sequence shown in SEQ ID NO:27.

12. The DNA molecule according to claim 23, wherein said DNA molecule comprises the nucleotide sequence shown in SEQ ID NO:26.

13. The DNA molecule according to claim 1, wherein said altered form of the NIM1 protein has an N-terminal truncation of amino acids corresponding approximately to amino acid positions 1-125 of SEQ ID NO:2 and a C-terminal truncation of amino acids corresponding approximately to amino acid positions 522-593 of SEQ ID NO:3.

14. The DNA molecule according to claim 13, wherein said altered form of the NIM1 protein comprises the amino acid sequence shown in SEQ ID NO:29.

15. The DNA molecule according to claim 14, wherein said DNA molecule comprises the nucleotide sequence shown in SEQ ID NO:28.

16. The DNA molecule according to claim 1, wherein said altered form of the NIM1 protein consists essentially of ankyrin motifs corresponding approximately to amino acid positions 103-362 of SEQ ID NO:3.

17. The DNA molecule according to claim 16, wherein said altered form of the NIM1 protein comprises the amino acid sequence shown in SEQ ID NO:31.

18. The DNA molecule according to claim 17, wherein said DNA molecule comprises the nucleotide sequence shown in SEQ ID NO:30.

19. The DNA molecule according to claim1, wherein said DNA molecule hybridizes under the following conditions to a nucleotide sequence selected from the group consisting of SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28 and SEQ ID NO:30: hybridization in 1%BSA; 520mM $NaPO_4$, pH7.2; 7% lauryl sulfate, sodium salt; 1mM EDTA; 250 mM sodium chloride at 55^{o}C for 18-24h, and wash in 6XSSC for 15 min. (X3) 3XSSC for 15 min. (X1) at 55^{o}C.

20. A chimeric gene comprising a promoter active in plants operatively linked to the DNA molecule according to anyone of claims 1 to 19.

21. A recombinant vector comprising the chimeric gene of claim 20, wherein said vector is capable of being stably transformed into a host cell.

22. A method of activating SAR in a plant, comprising transforming the plant with the recombinant vector of claim 21, wherein said altered form of the NIM1 protein is expressed in said transformed plant and activates SAR in said plant.

23. A method of conferring broad spectrum disease resistance to a plant, comprising transforming the plant with the recombinant vector of claim 21, wherein said altered form of the NIM1 protein is expressed in said transformed plant and confers broad spectrum disease resistance to said plant.

24. A method of conferring a CIM phenotype to a plant, comprising transforming the plant with the recombinant vector of claim 21, wherein said altered form of the NIM1 protein is expressed in said transformed plant and confers a CIM phenotype to said plant.

25. A host cell stably transformed with the vector of claim 21.

26. The host cell of claim 25, which is a plant cell.

27. A plant, plant cells and the descendants thereof comprising the chimeric gene of claim 19 which have a broad spectrum of disease resistance.

28. A plant, plant cells and the descendants thereof, wherein a NIM1 protein involved in the signal transduction cascade leading to systemic acquired resistance in plants is expressed in said transformed plant at higher levels than in a wild type plant.

29. A plant, plant cells and the descendants thereof of claim 27 or 28, wherein said plant is selected form the group consisting of gymnosperms, monocots, and dicots.

30. A plant, plant cells and the descendants thereof of claim 27 or 28, wherein said plant is a crop plant.

31. A plant, plant cells and the descendants thereof of claim 27 or 28, wherein said plant is selected form the group consisting of rice, wheat, barley, rye, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish,

spinach, asparagus, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane.

32. A method of conferring a CIM phenotype to a plant cell, a plant and the descendants thereof, comprising transforming the plant with the recombinant vector comprising the chimeric gene comprising a promoter active in plants operatively linked to the DNA molecule that encodes a NIM1 protein involved in the signal transduction cascade leading to systemic acquired resistance in plants, wherein said vector is capable of being stably transformed into a host wherein said NIM1 protein is expressed in said transformed plant at higher levels than in a wild type plant.

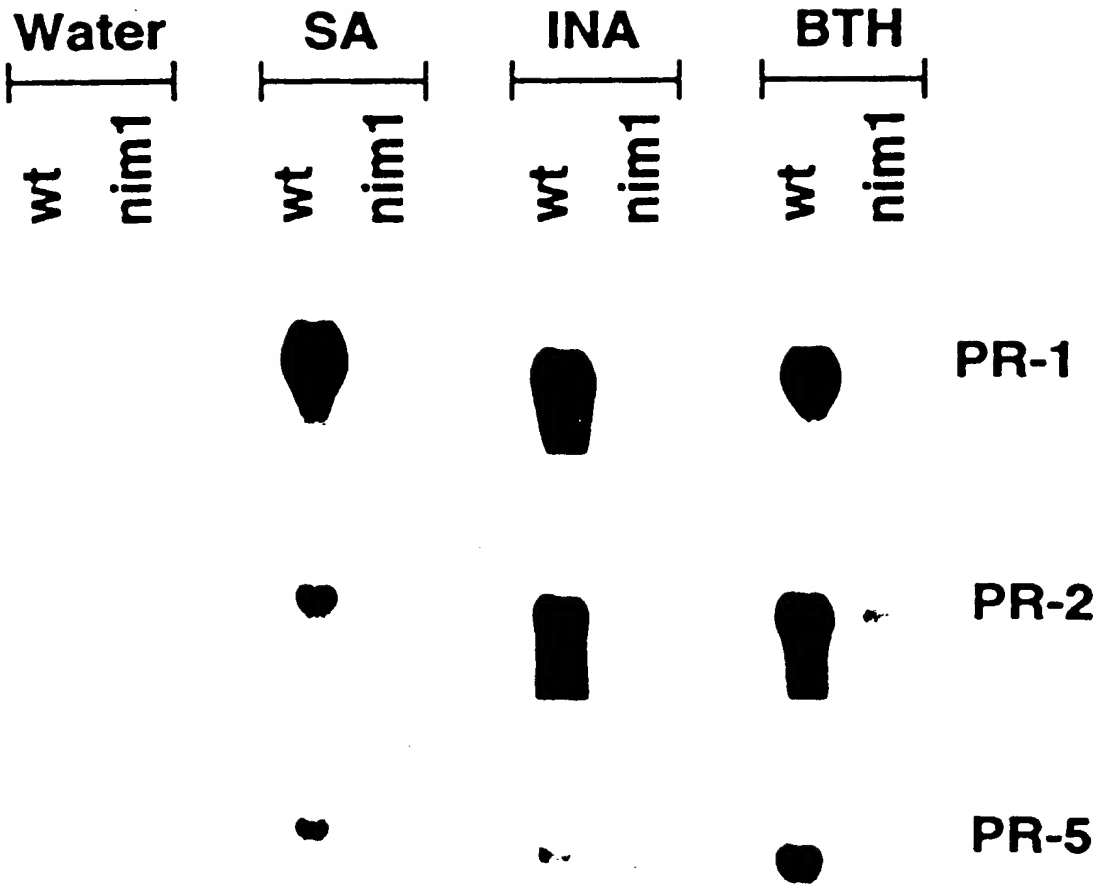
33. A method of activating systemic acquired resistance in a plant cell, a plant and the descendants thereof, comprising transforming the plant with the recombinant vector comprising the chimeric gene comprising a promoter active in plants operatively linked to the DNA molecule that encodes a NIM1 protein involved in the signal transduction cascade leading to systemic acquired resistance in plants, wherein said vector is capable of being stably transformed into a host, wherein said NIM1 protein is expressed in said transformed plant at higher levels than in a wild type plant.

34. A method of conferring broad spectrum disease resistance to a plant cell, a plant and the descendants thereof, comprising transforming the plant with the recombinant vector comprising the chimeric gene comprising a promoter active in plants operatively linked to the DNA molecule that encodes a NIM1 protein involved in the signal transduction cascade leading to systemic acquired resistance in plants, wherein said vector is capable of being stably transformed into a host, wherein said NIM1 protein is expressed in said transformed plant at higher levels than in a wild type plant.

35. Use of a transgenic plant or the descendants thereof comprising a chimeric gene according to claim 20 in an agricultural method. .

36. A commercial bag comprising seed of a transgenic plant comprising at least one altered form of a NIM1 protein or a NIM1 protein that is expressed in said transformed plant at higher levels than in a wild type plant together with a suitable carrier in an amount sufficient to act as a dominant-negative regulator of th SAR signal transduction

pathway, together with lable instructions for the use thereof for conferring broad spectrum disease resistance to plants.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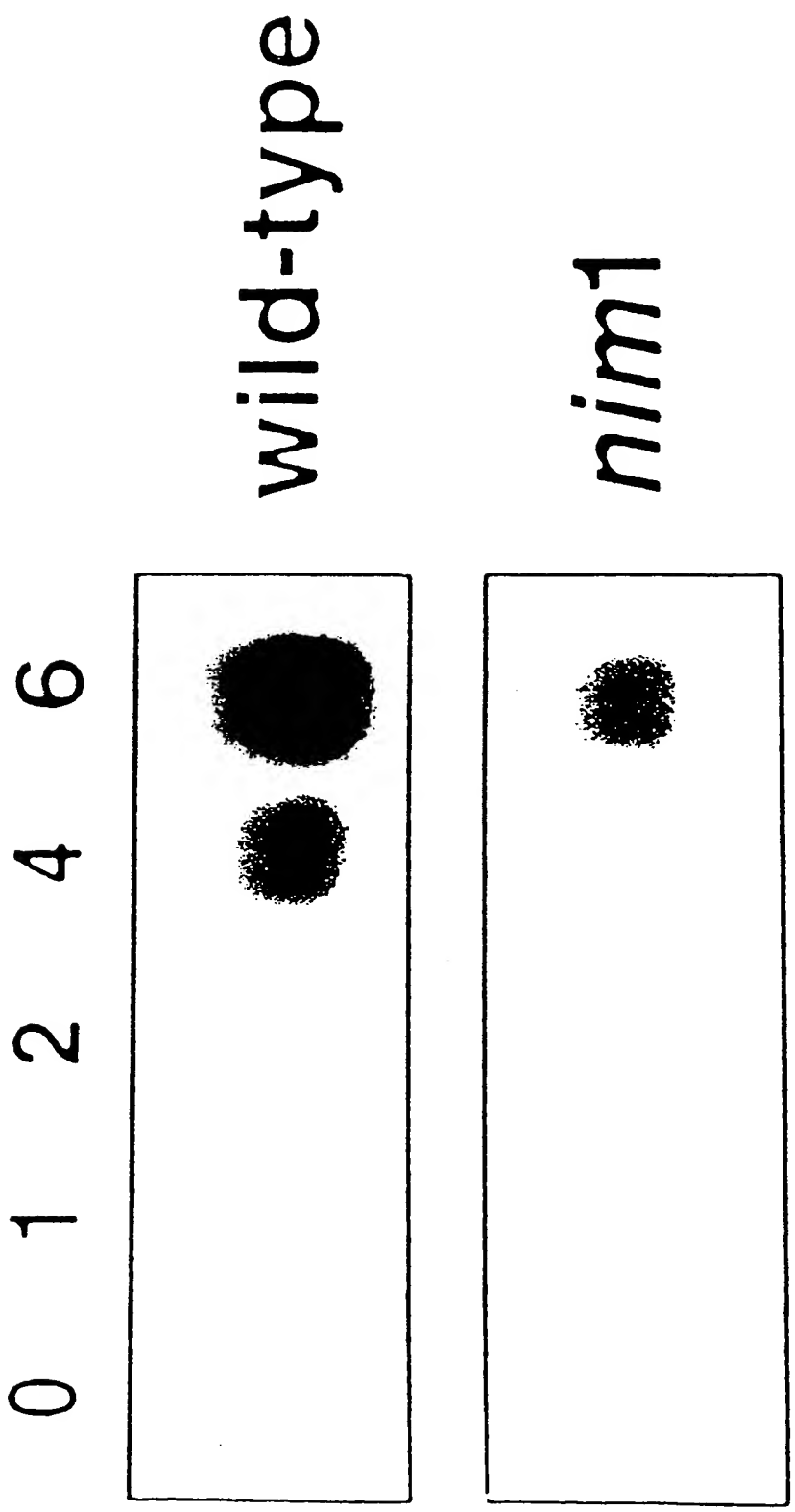

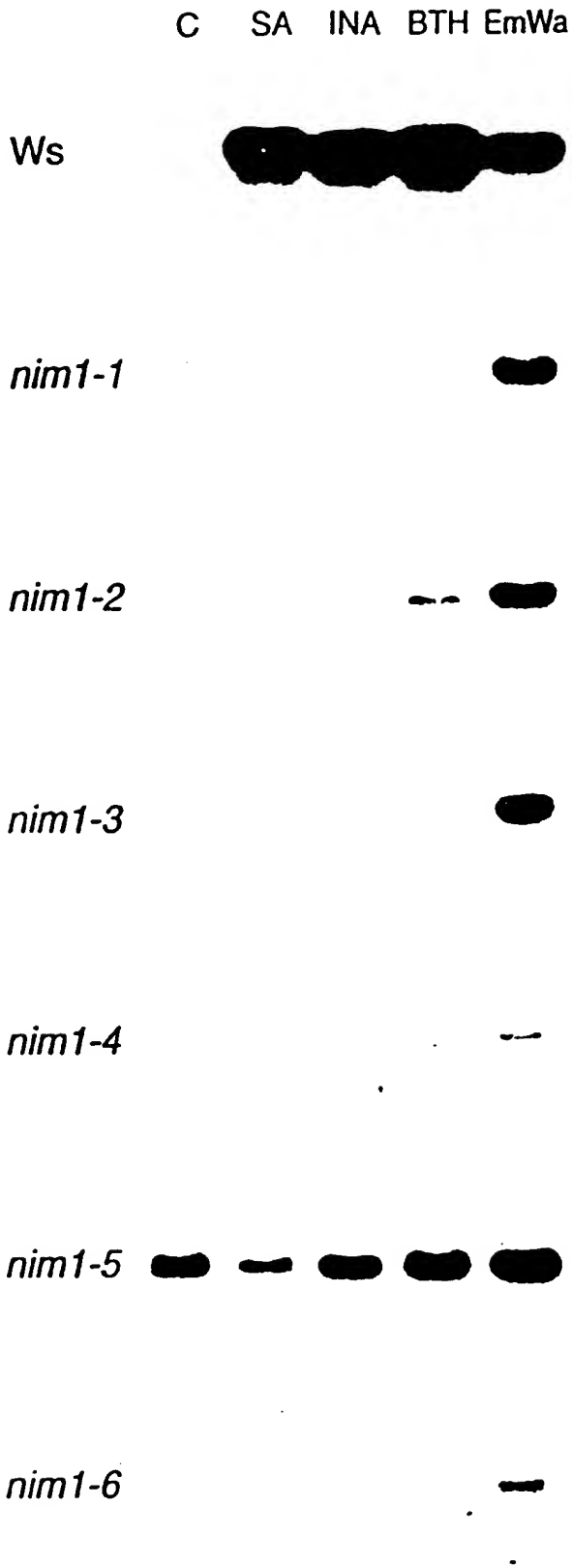
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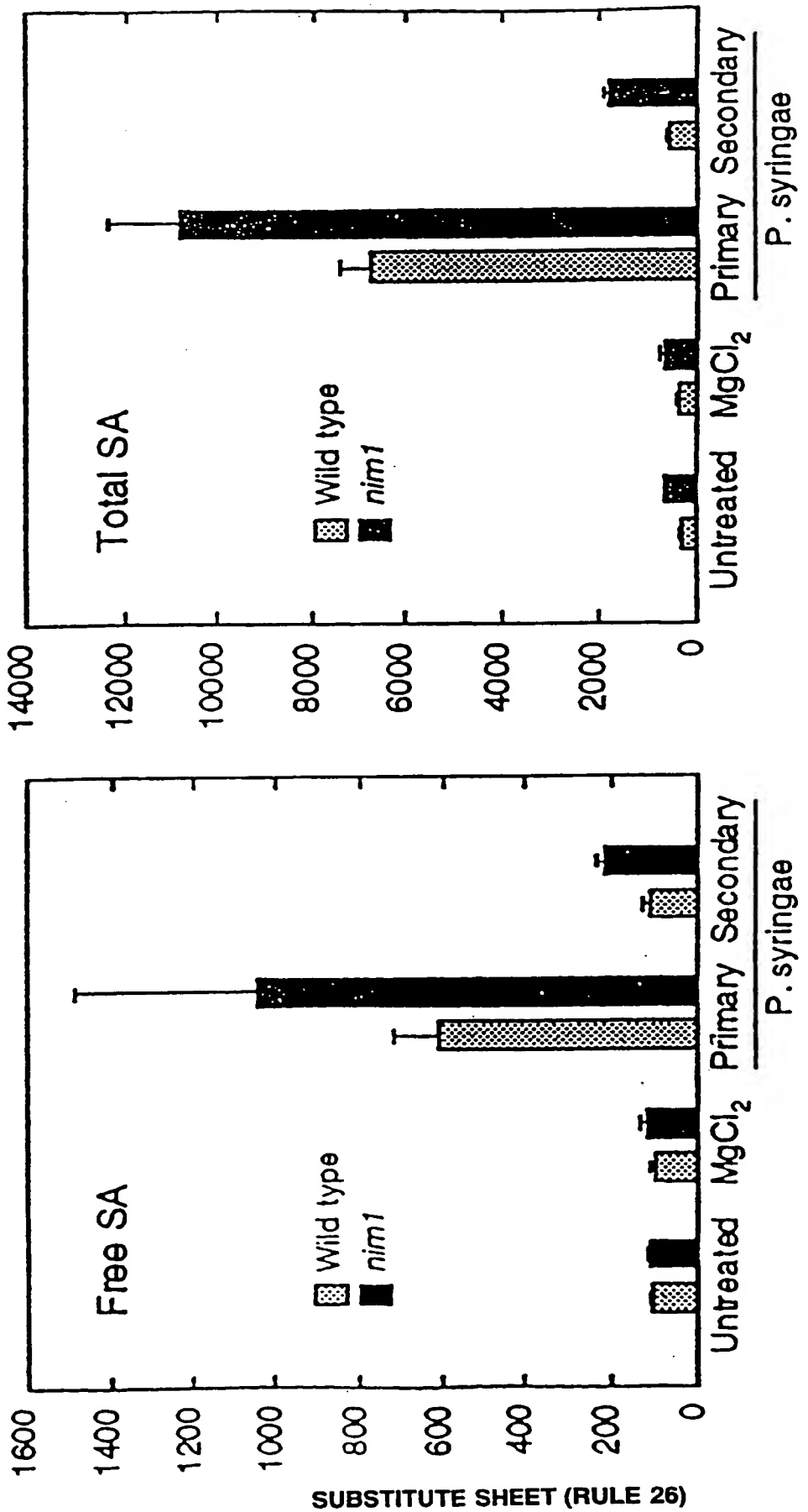
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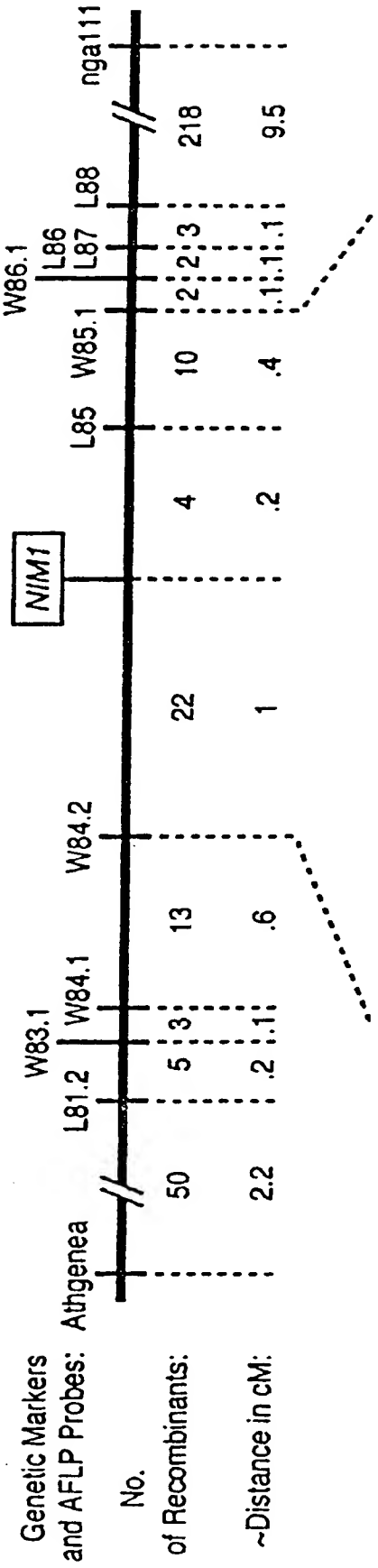
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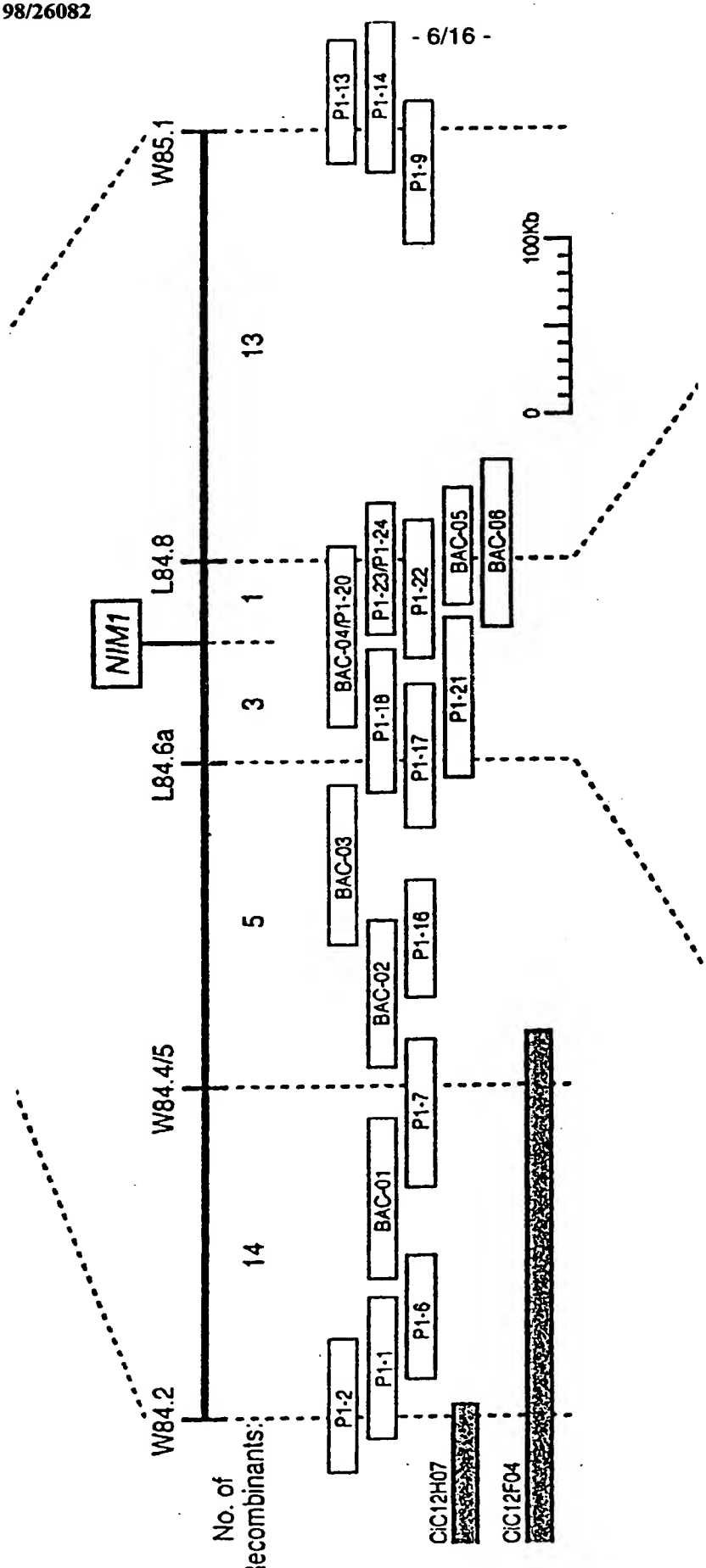
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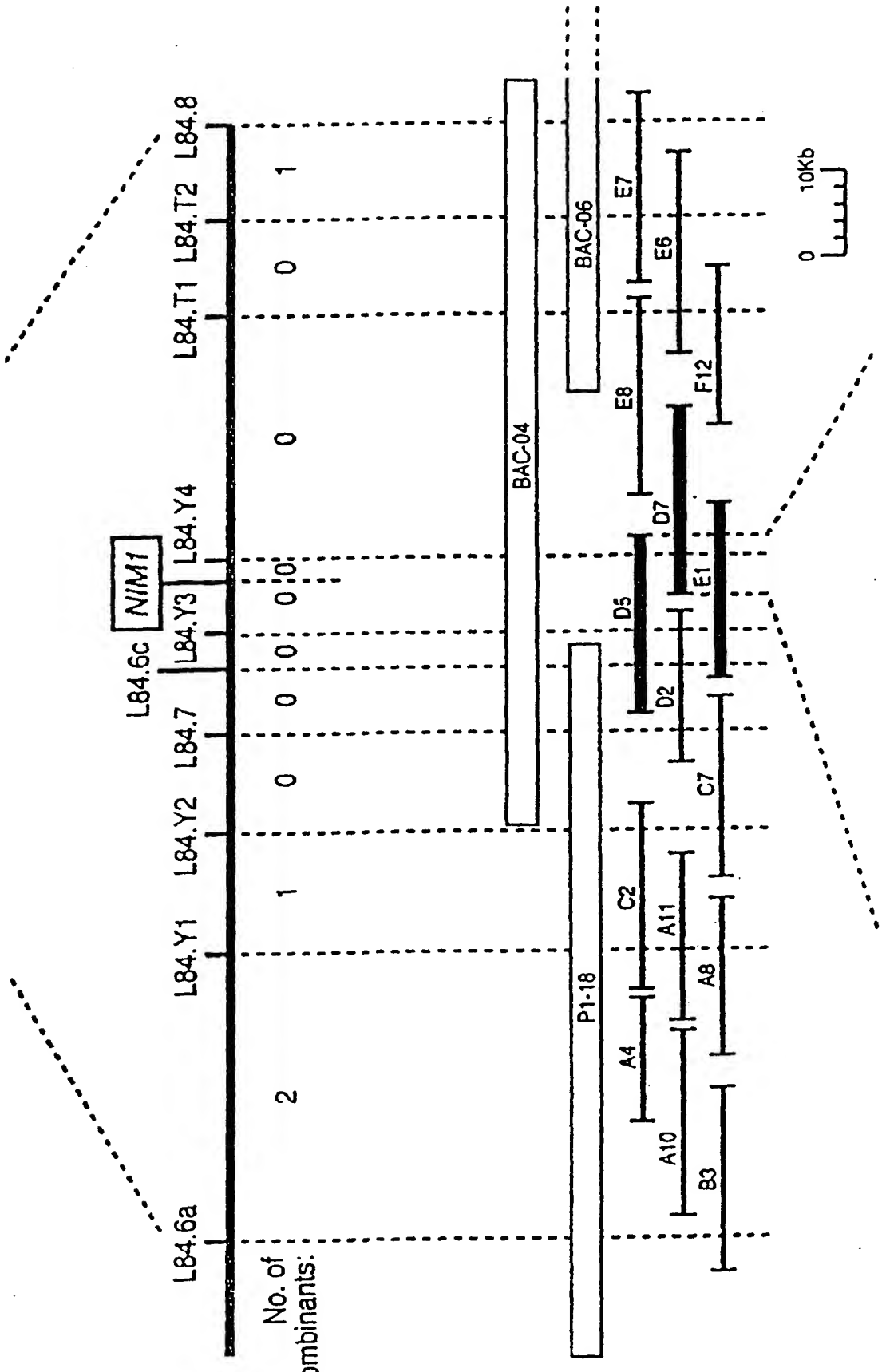
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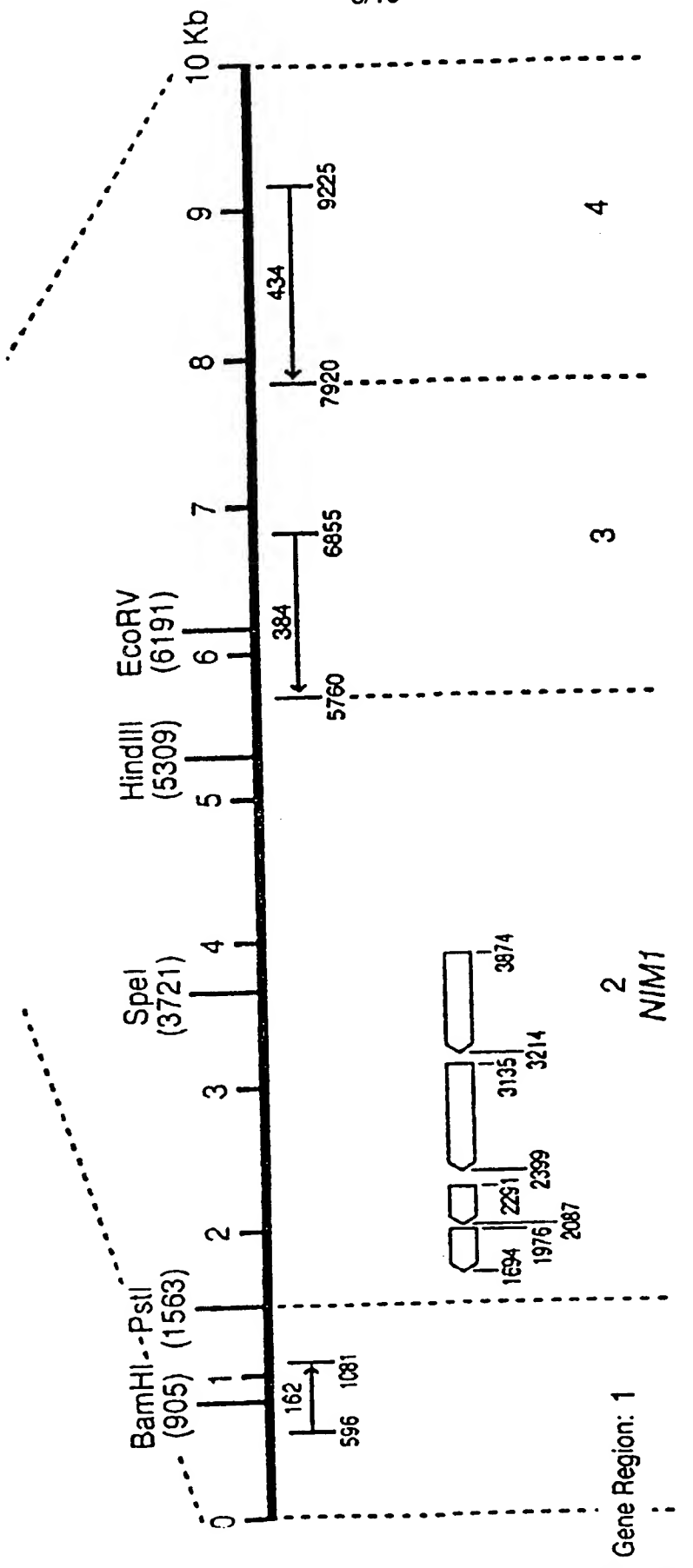

SUBSTITUTE SHEET (RULE 26)

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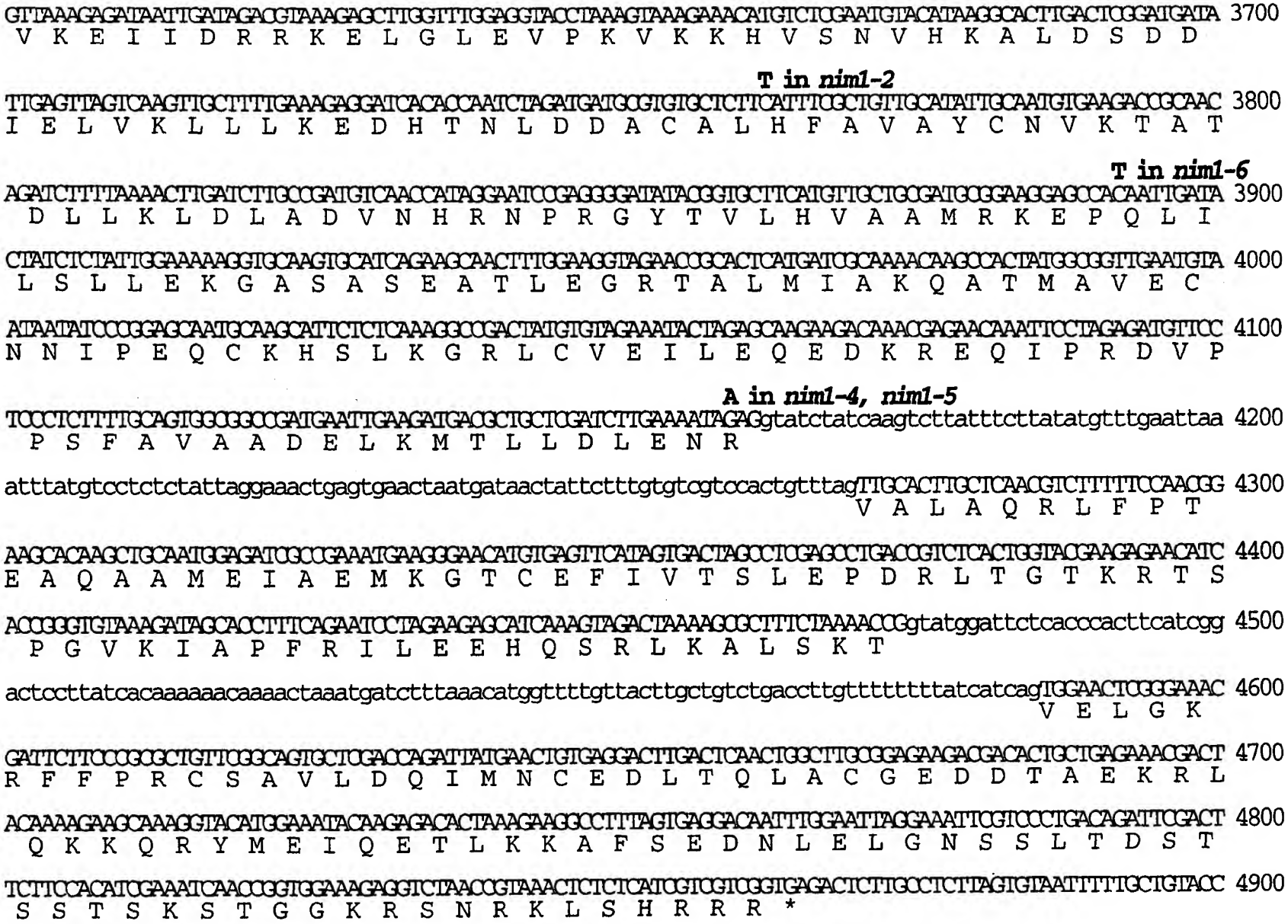

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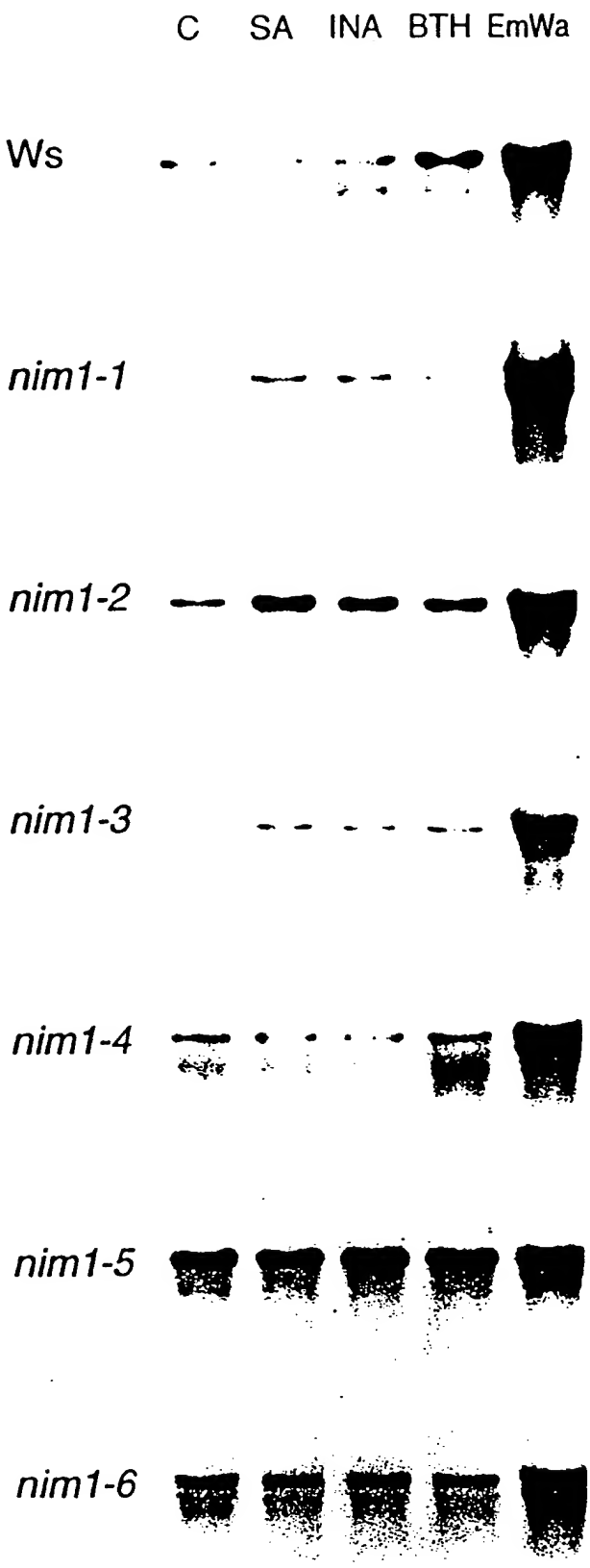
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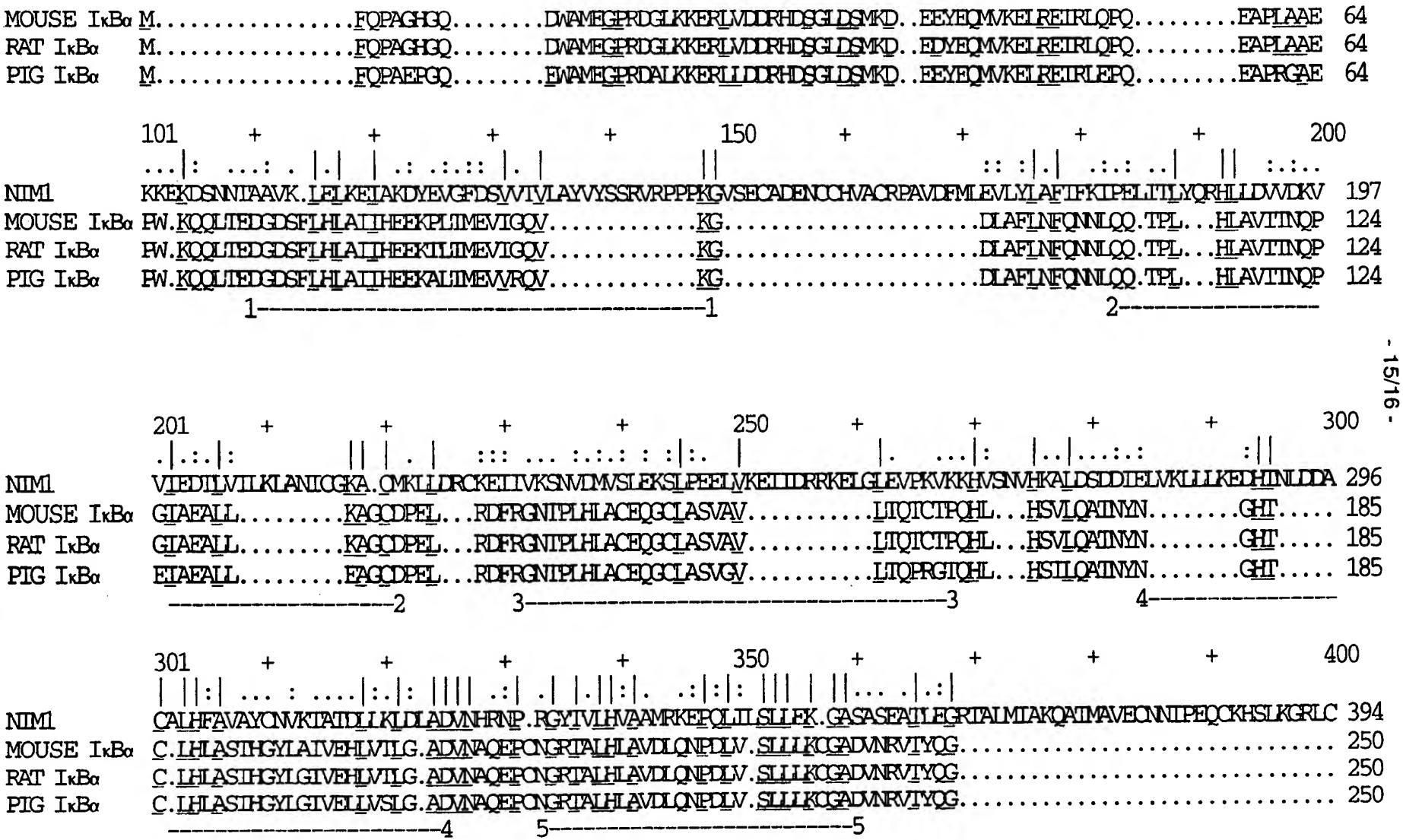

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INTERNATIONAL SEARCH REPORT

Interl. nal Application No
PCT/EP 97/07012

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N15/29 C07K14/415 C12Q1/68 G01N33/48
A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K C12Q G01N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 16077 A (CIBA GEIGY AG ;RYALS JOHN A (US); UKNES SCOTT J (US); DELANEY TERR) 21 July 1994 pages 7,8,15, pages 17-20, claims	28-34
X	NEWMAN, T., ET AL. : "GENES GALORE: A SUMMARY OF METHODS FOR ASSESSING RESULTS FROM LARGE-SCALE PARTIAL SEQUENCING OF ANONYMOUS ARABIDOPSIS cDNA CLONES" EMBL SEQUENCE DATA LIBRARY, 27 June 1994, HEIDELBERG, GERMANY, XP002059385 ACCESSION NO. t22612	19

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
18 March 1998	31/03/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

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Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DELANEY T P ET AL: "ARABIDOPSIS SIGNAL TRANSDUCTION MUTANT DEFECTIVE IN CHEMICALLY AND BIOLOGICALLY INDUCED DISEASE RESISTANCE" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 92, July 1995, pages 6602-6606, XP002034717 see the whole document ---	1-36
A	CAO H ET AL: "CHARACTERIZATION OF AN ARABIDOPSIS MUTANT THAT IS NONRESPONSIVE TO INDUCERS OF SYSTEMIC ACQUIRED RESISTANCE" PLANT CELL, vol. 6, November 1994, pages 1583-1592, XP002034718 see the whole document ---	1-36
A	HUNT M D ET AL: "Recent advances in systemic acquired resistance research -- a review" GENE, vol. 1, no. 179, 7 November 1996, page 89-95 XP004071969 page 93, right column; Fig. 2 ---	1-36
P,X	RYALS J ET AL: "THE ARABIDOPSIS NIM1 PROTEIN SHOWS HOMOLOGY TO THE MAMMALIAN TRANSCRIPTION FACTOR INHIBITOR IKB" PLANT CELL, vol. 9, March 1997, pages 425-439, XP002034719 pages 431, left column, 433, Table 3, Fig. 5 see the whole document ---	19
P,X	CAO H ET AL: "THE ARABIDOPSIS NPR1 GENE THAT CONTROLS SYSTEMIC ACQUIRED RESISTANCE ENCODES A NOVEL PROTEIN CONTAINING ANKYRIN REPEATS" CELL, vol. 88, 10 January 1997, pages 57-63, XP002034720 see the whole document ---	19
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INTERNATIONAL SEARCH REPORT

Intern. ial Application No
PCT/EP 97/07012

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 97 49822 A (CIBA GEIGY AG ;RYALS JOHN ANDREW (US); DELANEY TERRENCE PATRICK (U) 31 December 1997 pages 4,7,8,11,12,16; examples	1,19-35

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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